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Proceedings of the Sudden Oak Death Third Science Symposium

Quercus agrifolia

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Proceedings of the Sudden Oak Death Third Science Symposium

March 5-9, 2007 Santa Rosa, California

Susan J. Frankel, John T. Kliejunas, and Katharine M. Palmieri
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Abstract

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The Sudden Oak Death Third Science Symposium provided a forum for current research on sudden oak death, caused by the exotic, quarantine pathogen, *Phytophthora ramorum*. One hundred and seventeen submissions describing papers and posters on the following sudden oak death/ *P. ramorum* topics are included: biology, genetics, nursery, and wildland management, monitoring, ecology, and diagnostics.

Key words: Sudden oak death, *Phytophthora ramorum*, invasive species, tanoak, coast live oak.

Contents

Welcome, Overview, and Updates

Welcome to the Sudden Oak Death Third Science Symposium[1]

Susan J. Frankel[2]

On behalf of the United States Department of Agriculture (USDA)-Forest Service, Pacific Southwest Research Station and the California Oak Mortality Task Force, it is my pleasure to welcome you to the Sudden Oak Death Third Science Symposium. Looking back at the first sudden oak death science symposium held in Monterey in December 2002, it is amazing to see how far we have come in such a short period of time. It is hard to believe that at that point—a little over four years ago—we didn't even know *Camellia* was susceptible to *Phytophthora ramorum*.

It seems like yesterday when *P. ramorum* was first isolated and identified as the causal agent of sudden oak death. It was the summer of 2000, and Everett Hansen (Oregon State University) was in California for a visit. I remember my feelings of frustration as we drove through Marin County looking at the impacted forests; my mind was reeling with so many questions.

Why were so many coast live oaks (*Quercus agrifolia*) dying?
Was the same agent killing coast live oak and tanoak (*Lithocarpus densiflorus*)?
Was the same agent killing both the large and small trees?
Were the beetle attacks primary or secondary?
Why was the pattern of mortality so variable, starting at ridge-lines, mid-slope, or in canyon bottoms?

Everett nodded, smiled and said, "You know Susan, someday it will all make sense." Well, I am still waiting for that someday, but the unfolding events and research surrounding *P. ramorum* is an amazing story of discovery. It is fascinating to ponder the current unknowns as well as the key findings that have brought us to where we are today.

Since it is often the questions that drive the discoveries, I challenge you to carefully consider what is presented at this symposium. Throughout this week, ask yourself what doesn't make sense. Ask yourself what doesn't fit with your observations. Your questions have been, and will continue to be, the key to our progress.

How many times have you heard, *P. ramorum* kills trees by inciting girdling cankers? Yet, infected trees often die before they are completely girdled. How can this be? Something about this answer has never seemed quite right. Recognizing this discrepancy, research has recently been conducted to better understand how *P. ramorum* kills trees. Without giving away too much, I will say that new findings

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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of *P. ramorum* and its spread in wood are unraveling old theories and introducing new concepts.

So I am on a hunt for new, key questions. Where is the sudden oak death story incomplete? We need your assistance. On Friday we are holding a research needs assessment session to identify and prioritize future research. What problem are you struggling with that needs to be addressed by new or additional research? What pieces of the puzzle do you see not fitting?

As the story unfolds, we face increasingly challenging questions as well as evidence that is sometimes hard to face. How well are sudden oak death treatments working? Can *P. ramorum* be eradicated, or its spread slowed, in nurseries or forests? These are hard problems to solve that require courage, dedication, and the ability to continually adapt to new realities.

Often the greatest opportunity for progress comes in honest dialogue, constructive criticism, and in continuing to wonder and ask good questions. To support a collaborative environment, the format for this symposium has been designed to bring all disciplines of sudden oak death and *P. ramorum* together.

I thank all of you for being here in spite of budget problems and winter weather. It is exciting to see this gathering of the world's "SOD Squad."

Acknowledgments and Thanks

The conference committee thanks the North American Plant Protection Organization for organizing and hosting Monday's "Risks to conifers" session.

Thank you to the University of California Integrated Hardwood Range Management program for subsidizing student registrations.

Thank you to Alan Kanaskie, Jack Marshall, Reno Franklin, Eric Wilder, Yana Valachovic, Chris Lee, Ted Swiecki, Chris Perry, Caerleon Safford, Jennifer Parke, Dave Rizzo, Alan Inman, Russ Bullock, Kerry Britton, Jonathan Jones, Joan Webber, and Stephen Hunter for your contributions to the symposium field trip and research needs assessment session. Thank you to Katie Palmieri and Janice Alexander for organizing this symposium.

Rethinking *Phytophthora*—Research Opportunities and Management[1]

Everett Hansen[2]

Introduction

It was the second week of June, 2000, the hottest weather on record in San Francisco - hardly *Phytophthora* weather. But it was that week, at China Camp State Park, that Dave Rizzo and colleagues collected the bark samples from bleeding cankers on coast live oaks that finally moved sudden oak death (SOD) from the "cause unknown" category to "*Phytophthora* disease" (Rizzo and others 2002). Research progress has been dramatic in the last seven years. Think of the advances in *Phytophthora* genetics, capped by publication of the complete genome sequence, and its applications in diagnostics and population genetics. Think of the discovery of other *Phytophthora* species with similar life styles to *Phytophthora ramorum*, but apparently indigenous to the same forests. This was unexpected, and is stimulating a resurgence in *Phytophthora* taxonomy worldwide. Think of the substantial efforts in nursery disease research to understand the spread and survival of *P. ramorum* in this intensely manipulated environment that are leading to development of "best management practices," and fewer and fewer infested nurseries. It would be reasonable, and comforting, to use this time at the beginning of this Sudden Oak Death Third Science Symposium to reflect on our past accomplishments. They are many. But somehow I find myself more impressed with the challenges of the future. I want to highlight some of the new and exciting work to be presented at this meeting, work that is opening new research directions, and to consider the daunting and escalating management challenges that this disease is forcing on us.

The map of the distribution of SOD in the West hasn't changed much since the first versions went on-line in 2001. A couple of new infested counties were added in California, and new disease spots in Humboldt and Mendocino Counties and in the Big Sur are troubling, but western California hasn't turned red. The alarm comes at a finer scale. The small patches of dead trees have multiplied, and in more and more areas the patches are now hillsides of mortality. Tanoak mortality levels exceed 80 percent on many plots, and local extinction of the species seems increasingly probable.

At the same time, it is becoming clear that coast live oak is less susceptible than tanoak; perhaps the disease will prove manageable in oak woodlands. One of the breakthroughs in research has been the realization that *P. ramorum* causes very

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different diseases on its many different hosts (Davidson and others 2003, Hansen and others 2005). Unfortunately we still have little insight into the long-term impacts of the pathogen on the many native dieback and leaf blight hosts growing in affected forests.

I have organized this presentation in three parts, two highlighting research that I find particularly exciting, and a final section challenging our disease management (in-) actions:

- Look Up!
  Phytophthora epidemiology
- What's so SUDDEN about SOD?
  Pathogenesis
- Fight Them on the Beaches
  Disease Management

Phytophthora Epidemiology

Phytophthora ramorum is an aerial *Phytophthora*. The pathogen does get into streams and the soil and persists there, and it can infect roots under some conditions at least. There is much left to discover about its behavior below ground. But in the forest especially, the action is above ground.

Phytophthora ramorum is spreading in three very different patterns in the forest: 1) local intensification of disease is driven by sporangia splashing and dripping downward from high infections; 2) the pathogen is spotting across the landscape, initiating new disease foci, probably by turbulent dispersal of sporangia in dry air; and 3) infected plants are moving very long distances in the nursery trade, threatening new forest infestations in distant parts of the country and the world. Intensive efforts to cut off the nursery pathways are having increasing success. Rain splash dispersal is well documented now in several forest types (Davidson and others 2005; Davidson, Patterson and Rizzo, in press). It is important at distances out to 10 or 20 m from a source. Turbulent dispersal is the new story for *P. ramorum*.

This pathogen is well adapted for aerial spread. Both California bay (in California) and tanoak are important hosts, supporting sporulation on leaves and twigs. Chlamydospores are formed inside infected leaves, but in the lab at least, they also form on the surfaces of leaves and twigs. Certainly they are transported as trees defoliate, and are released into the soil as leaves decompose (Fichtner and others 2007). Are chlamydospores that formed on leaf surfaces free and effective inoculum spread by rain splash? We have much to learn about chlamydospores as infection propagules. They are built to withstand harsh conditions. What triggers them to germinate?

Deciduous sporangia are the hallmark of an aerial *Phytophthora*. They form on leaves and twigs, and are readily dislodged by rain drops. Splash dispersal is an important part of SOD epidemiology (Rizzo and others 2005). But how to explain the observed dispersal across hundreds and even thousands of meters? This is well beyond the normal range of splash dispersal. Storm driven rain has been invoked, but why not turbulent dispersal? There is ample precedent in related pathogens.

Blue mold of tobacco, caused by the oomycete *Peronospora tabacina*, and late blight of potato, caused by *Phytophthora infestans*, provide well documented model systems. Both are characterized by deciduous sporangia formed on infected leaves in the canopy of the crop. The sporangia form in relatively cool, moist air at night, and are released into turbulent air currents as the atmosphere above the crop warms and dries in the daytime. Clouds of sporangia may be released from infested crops, lofted on rising turbulent air, and transported for hundreds of meters, even thousands of kilometers in the case of blue mold, before settling out of the air, or more commonly being washed out by rain (Aylor 1999, Aylor and others 1982, LaMondia and Aylor 2001, Zwankhuisen and others 1998). Infection at a great distance is a rare event, but with high inoculum loads, susceptible hosts, and favorable winds with appropriate atmospheric conditions, it happens regularly in potato and tobacco.

Is this dry air turbulent dispersal important for SOD? The evidence is yet incomplete, but it seems likely: 1) Tanoak trees are infected in the upper canopy, and deciduous sporangia are formed (Rizzo and others 2005); 2) The pattern of new infections in the Oregon eradication area mirrors a classical turbulent transport dispersal gradient (Gregory 1968) (Fig. 1); and 3) Microsatellite genotyping shows that new infections (up to 4 km distant) in Oregon are coming from inside the infested area, not from outside (Prospero and others 2007). When might turbulent dispersal occur? Let us suppose it takes two days with very high humidity and temperatures 15°C for sporangia to form on leaves (Englander and others 2006), followed by a short period of

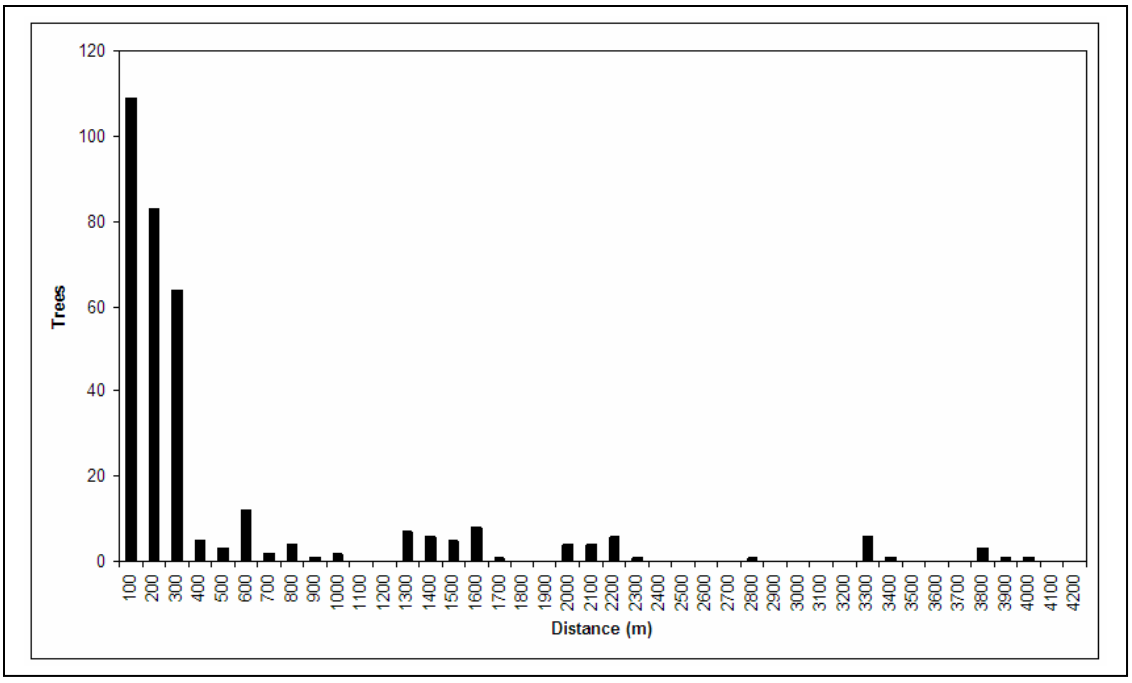

Figure 1—Distance between tanoak trees killed by *P. ramorum* and the nearest tree killed by the pathogen in a previous year.

warming and drying to snap the sporangia off of their stalks and establish rising air currents. Add a gentle breeze and they are on their way. Gravity, or cooling air, or rain will bring them down. Yes, sporangia are vulnerable to drying and UV light, but the potato and tobacco pathogens, at least, survive even rather harsh conditions for an hour or so (Bashi and Aylor 1983, Englander and others 2006, Mizubuti and others 2000). I imagine a warm wet period in Curry County in the spring, with new, vulnerable growth on the trees. With clearing weather, rain is replaced overnight by dew and fog. The fog breaks up as temperatures rise and the day progresses, lofting the sporangia. Cooling air settles downslope in the evening and the fog forms again, providing moisture now for germination of sporangia on tender new leaves.

Phytophthora ramorum Pathogenesis

What is so sudden about "sudden oak death?" We know now that it takes at least a year from initial infection of a tree to death, even in the very susceptible tanoak (McPherson and others 2005). Death isn't really a sudden process in trees, yet the entire crown often turns red all at once. A tree is green one season and red the next. The culmination of some of the earliest SOD work, and the initial results of new research directions are helping us understand just how, and why, infected trees are killed by this disease.

Oak bark beetles were among the first suspects as causal agents of SOD. We now know that they are secondary invaders, attracted to and breeding in trees already made attractive by *Phytophthora ramorum* (McPherson and others, this Proceedings). The pathogen can and does kill oaks without the aid of beetles, but it usually doesn't get the chance. Oak bark beetles are ubiquitous and often numerous, and find diseased trees quickly. Their galleries, and the staining fungi that accompany them, quickly kill the inner bark and living cells in the sapwood, further blocking water movement in the trunk, and leading to death of the crown. Trees attacked by bark beetles after *P. ramorum* infection die more quickly than trees infected by the pathogen alone (McPherson and others 2005).

Oak ambrosia beetles also contribute to the "sudden" appearance of the disease. These beetles burrow into the sapwood of trees to excavate their egg galleries. Like the bark beetles they are secondary attackers, and like the bark beetles they carry pathogenic fungi with them that hasten death of cells and decay the wood of oaks and tanoaks. Tanoak appears to not form heartwood. Sapwood, and ambrosia beetle galleries, extend right through the tree. One aspect of "sudden" in SOD is sudden breakage and collapse of the tree. This results in large part from the galleries and associated wood decay of the ambrosia beetles.

Back to the primary agent of SOD, *P. ramorum*. How does it kill the tree? The new part of the story is shifting the focus from the iconic bleeding cankers in the bark to behavior of the pathogen in the sapwood beneath the bark cankers.

The classical view of *Phytophthora* in trees focuses on bark cankers, with trees girdled by necrosis of the inner bark, the phloem and the cambium. There have been a few observations of other *Phytophthora* species in sapwood of their various hosts (Davison and others 1994, Oh and Hansen 2007), but they attracted little attention. It was noted early on that the sapwood behind *P. ramorum* bark cankers was sometimes discolored. Both Dave Rizzo and I isolated the pathogen from sapwood on occasion but didn't follow up. That has now changed. Two research groups, led by Anna Brown in England (Brown and Brasier 2007), and Jennifer Parke in this country (Parke and others 2007), have demonstrated that sapwood colonization is a regular and important feature of *Phytophthora* pathogenesis in several tree/*Phytophthora* combinations, including tanoak/*P. ramorum*. The British are suggesting that not only does *P. ramorum* survive from year to year in the sapwood, but that it also spreads up and down the tree in the xylem, creating new bark cankers, and bleed spots, from the inside out.

Work in Oregon has moved on to understanding the impact of xylem colonization on pathogenesis. *P. ramorum* was monitored in wood by isolation, microscopy, and PCR. Hyphae were seen in various cell types, but especially in xylem vessels. Chlamydospores were also present in vessels, and in many cases tyloses had ballooned through the vessel bordered pits from adjoining parenchyma cells. The net results were visible obstructions within the xylem vessels, and impeded water flow (Parke and others 2007).

Sap flow was monitored in green, infected trees and in uninfected trees. There was a significant reduction in xylem water transport in infected trees, before crown symptoms were evident. What other impacts might *P. ramorum* hyphae in the xylem have on host physiology?

Dan Manter, United States Department of Agriculture (USDA)-Agricultural Research Service (ARS) in Fort Collins, Colorado, has been working with the elicitins formed by *P. ramorum* (Manter and others this Proceedings). Elicitins are a class of low molecular weight proteins produced by *Phytophthora* species. They function as sterol transport proteins, damaging host cell membranes and carrying the released sterol molecules back to the mycelium (Bonnet and others 1996, Mikes and others 1998). *Phytophthoras* require exogenous sterols for reproduction. Each *Phytophthora* species produces its own specific elicitins. Dan has isolated the *P. ramorum* elicitins, and compared their effects on host tissues with the effects of the intact pathogen (Manter and others 2007). Both *P. ramorum* infection, and elicitin uptake from purified culture filtrates, trigger early reductions in carbon assimilation, stomatal conductance, and water transport. Elicitins are evidently key players in pathogenesis (Ricci 1997).

Managing *P. ramorum* in Western Forests

"Fight them on the beaches, or let the new order begin"
Professor Hal Mooney, Stanford University

Dr. Mooney's pronouncement was directed at the worldwide ecological threat from invasive organisms in general, but it encompasses the range of disease management situations forest pathologists face with *P. ramorum* very well. The fighting is fierce and increasingly successful in the nurseries, with the quarantine and eradication

regulations designed to keep SOD from spreading to new areas around the country and the world. In Oregon, the forest epidemic is being confronted acre by acre, with a local eradication campaign designed to halt the further spread on this particular front. The situation in California is much more complex, however, with the pathogen well established in many areas and still spreading in others.

In 2000, within a few months of identification of a new *Phytophthora* as cause of SOD, Oregon had begun early detection surveys, and in 2001, within weeks of locating the initial SOD infestations in the state, the eradication effort had begun. Despite early detection and prompt action, six years later we have not yet succeeded in eradicating the pathogen from its Oregon beachhead (Kanaskie this Proceedings). The pathogen is successfully neutralized on treated sites, but it continues to jump to new areas ahead of our eradication efforts. The net effect is a more or less stable infestation; we have, at least temporarily, contained the pathogen by preventing a dramatic increase in inoculum. The relative success of the Oregon effort is evident when compared to the explosive spread of SOD in Humboldt County, California (Fig. 2). The disease was first detected at about the same time, across roughly the same area in the two counties.

California lacks an articulated overall strategy for ramorum management. Without aggressive action, there is little to do except watch the “new order,” the aftermath forest without tanoak, develop. The “no action alternative” future is increasingly clear. We don’t need to guess any longer about the possible ecological impacts of *P. ramorum* on California forests. Tanoak is rapidly disappearing from expanding areas of Marin, Sonoma, Santa Cruz, Humboldt and Mendocino counties, and the Big

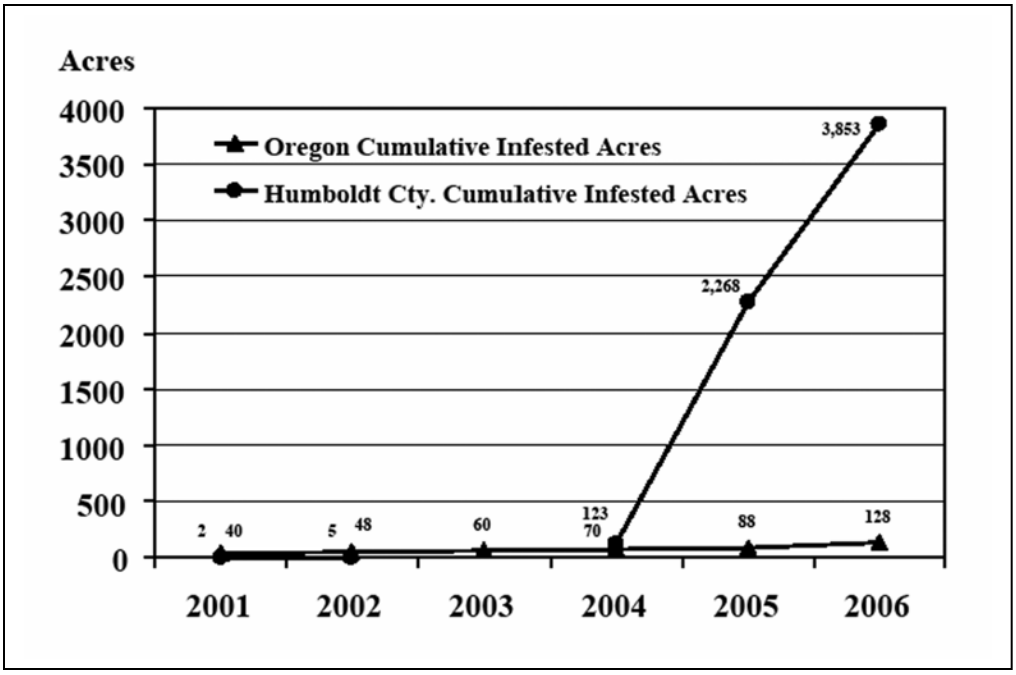

Figure 2—Increase in tanoak forest area affected by *P. ramorum* from 2001-2006, in Humboldt county, California and Curry county, Oregon.

Sur region of Monterey County. The epidemic has created a valuable ecological laboratory for the study of the impacts of invasive disturbance on ecosystem function, and several labs are taking advantage of the opportunity (Rizzo and others 2005, Maloney and others 2005). Exciting work is being done. There is still a lot of tanoak left in other parts of the state, however, and good opportunities remain to try to stop the further spread of the pathogen, especially to the north and east.

One obstacle to a concerted SOD control program is ambivalence about the importance of tanoak in western forests. Tanoak has essentially a negative economic value in the timber industry. It is most often viewed as an aggressive weed, competing with much more valuable conifers for growing space. Its ecological values need to be articulated more clearly, including its important roles as a mast producer (important to indigenous peoples as well as to wildlife), and as an early colonizer and stabilizer of disturbed sites.

It is also important to remember the economic impacts of regulatory actions triggered by further spread of SOD. These direct losses and indirect costs are already documented for the horticultural nursery industry. Douglas-fir is another host for *P. ramorum*, unlikely to be seriously impacted in commercial forests, but still subject to unpredictable international quarantine regulation.

It comes down to a simple question: "How serious are we about controlling SOD?" We have learned enough in the last six years to make that a manageable question, worthy of site specific consideration. It need not be all or nothing. The question should be answered separately for coast live oak forests, where managing California bay and judicious use of phosphonate may prove sufficient to save large numbers of trees in critical landscapes. The western Sierra Nevada in California is considered at lower risk to SOD because of climate. That suggests that the disease won't spread as fast there, and that aggressive eradication efforts have a better chance of success. Are we surveying regularly for early detection, and most importantly, are we administratively ready for prompt action when the pathogen is confirmed in the Sierra? Who will do what, and who is going to pay for it? The same questions of responsibility and readiness hold for the high risk forests of the eastern U.S.

Are we serious enough about stopping the northward spread of SOD to try a landscape level approach? The answer has been "no," in California, but that can change. Why aren't we using aerial applications of phosphonate to stop the advance of this disease? They do in Australia, against a related pathogen (Hardy and others 2001). A reasonable answer would be "because we don't know if it will work on *P. ramorum* and tanoak." But then why aren't we trying to find out if it will work? What about host-free barriers across the landscape? Dramatic? Certainly, and not to be undertaken lightly, but certainly worthy of careful consideration and reasoned debate.

Finally, I want to highlight another aspect of the SOD phenomenon. New models of research support and collaboration have been generated that should continue to produce benefits for years to come. The several branches of the Washington Office of the USDA-Forest Service have been central to the program. They allocated money to get things started, and worked with California politicians and Congress to generate substantial and continuing support. The Pacific Southwest Research Station, and Pacific Southwest Region of the Forest Service have been creative and unstinting

coordinators of an ever expanding research and disease management program. My California university colleagues especially have leveraged seed money from the Forest Service into National Competitive Grants from the National Science Foundation (NSF) and other programs, the Joint Genome Initiative, and Private Foundations.

New patterns of cooperation and collaboration have been forged, between institutions and agencies, between states, and internationally. The participation of our European colleagues has been invaluable in both the science and the regulation of *P. ramorum*. The California Oak Mortality Task Force is unique and invaluable. In Oregon, the convergence of skills and commitment and camaraderie in our small team is the highlight of my career. Thank you all.

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Eradication of *Phytophthora ramorum* From Oregon Forests: Status After 6 Years[1]

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Abstract

Sudden oak death (SOD), caused by *Phytophthora ramorum*, was first discovered in Oregon forests in July 2001. Since then an interagency team has been working with landowners to eradicate the pathogen by cutting and burning all infected and nearby host plants. During the first two years of the eradication effort, all host vegetation within 15 to 30 m of infected plants was destroyed. In recent years this distance has been increased to at least 100 m, reflecting recent research findings on spread of the pathogen. On private land, all tanoaks (*Lithocarpus densiflorus*) are injected with herbicide prior to cutting in order to prevent stump-sprouting following cutting and burning. On most sites, follow-up treatments are necessary to destroy residual host material and stump sprouts that may harbor the pathogen. Following burning most sites are planted with non-host or conifer seedlings.

During the first four years of the eradication effort, the number of new infested sites and infected trees remained steady or decreased each year, indicating modest success at containment and eradication. That trend ended in 2005 when the number of new infected trees and the amount of infested area began increasing (fig. 1). In 2006 we discovered 36 new infested sites (143 infected tanoak trees). Two of the new sites occurred outside of the quarantine zone: one was 1.5 km to the east and the other 2.5 km to the west of the boundary. Each of these sites was more than 3 km from the nearest other infested site. Most of the other new sites were small (less than 0.4 ha) and scattered near the center of the quarantine zone along the North Fork Chetco River and its tributaries (fig. 2). The largest new site covered 4 ha and contained more than 40 infected trees. In addition to the new sites, six existing eradication sites were expanded to include infected trees that were found near their perimeters. We attribute this uncommon and unexpected amount of disease expansion to two consecutive years of unusually wet spring and early summer weather. The 2006 weather, in particular, appeared to favor long distance spread of *P. ramorum*.

Eradication treatments have been completed or are underway on approximately 410 ha of forest land, at a cumulative cost of $1.5 million. Eradication costs have been funded primarily by grants from the United States Department of Agriculture - Forest Service to the Oregon Department of Forestry to assist private landowners with the eradication treatments. There is no compensation to landowners for the value of timber or other resources lost as a result of the eradication treatments. The area treated for eradication is distributed among landowner groups as follows: private industrial (72 percent, one owner); non-industrial private forests

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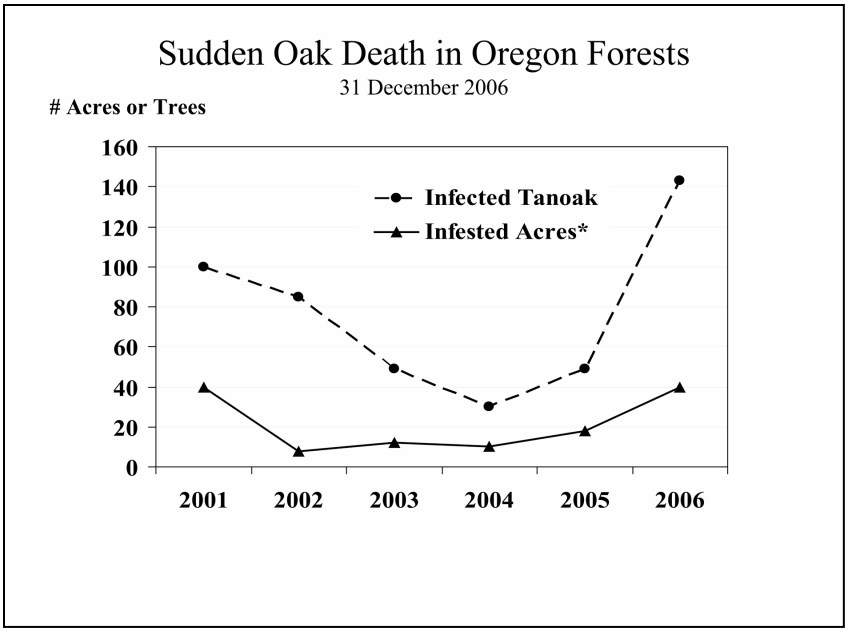

Figure 1—*P. ramorum* in Oregon forests: trend in number of new infected tanoak trees and new areas infested each year (not cumulative). The estimated number of infested acres includes new infested sites and expansions of eradication sites existing in previous years.

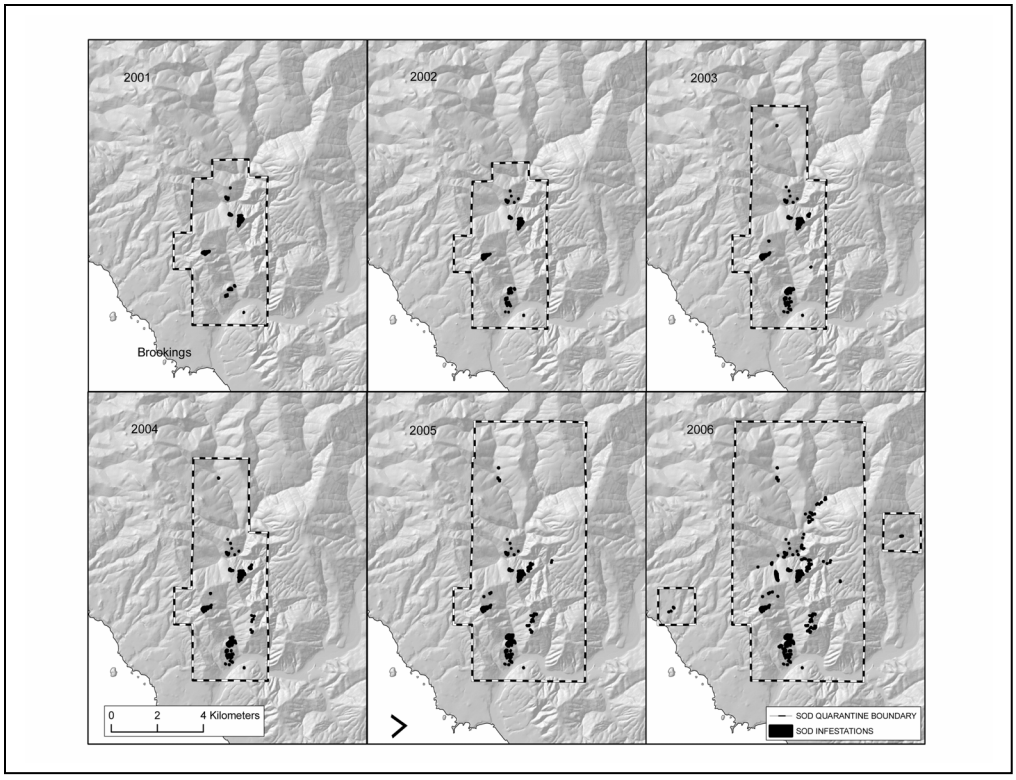

Figure 2—Location of forest sites infested with *P. ramorum* in southwest Oregon, 2001 through December, 2006. All infested sites have received eradication treatments. The estimated number of infested acres includes new infested sites and expansions of eradication sites existing in previous years.

(18 percent, five owners); rural-residential (6 percent, 17 owners); United States Department of Interior - Bureau of Land Management (3 percent); USDA - Forest Service (<1 percent), and; and State of Oregon (<1 percent).

To date we have had modest success at eradication and very good success at limiting spread and containing the pathogen to a relatively small area. Several of the treated sites appear pathogen-free two years after treatment, and the pathogen has not expanded from these sites. Repeated aerial surveys, ground surveys, and stream monitoring throughout southwest Oregon have failed to detect the pathogen in forests beyond the quarantine area near the town of Brookings. Extensive surveys from northern California to the Columbia River also have failed to detect the pathogen anywhere except in the Curry County quarantine area.

The success of the program thus far can be attributed to a number of factors including: thorough aerial, ground, and stream monitoring surveys that facilitate rapid detection and response to the disease; consensus among the interagency pathologists involved; adequate federal funding, and; cooperation from affected landowners on private and federal lands. Continued and improved early detection surveys, rapid application of eradication treatments, and landowner assistance are critical to the long-term effectiveness of the containment and eradication program.

Key words: *Phytophthora ramorum*, sudden oak death, Oregon, eradication.

Status of *Phytophthora ramorum* and *P. kernoviae* in Europe[1]

Joan F. Webber[2]

Abstract

Following the recognition that *Phytophthora ramorum* (the cause of sudden oak death in the U.S.) was present in Europe as well as America, emergency European Community (EC) phytosanitary measures were put in place in September 2002 to prevent spread of *P. ramorum*, and also to stop introductions of the pathogen from elsewhere. A 3 year European project then started in 2004 to assess the risks posed by *P. ramorum* to trees and environmentally important habitats in Europe. The project – 'Risk Analysis of *Phytophthora ramorum'*, known by the acronym RAPRA, involves eight research organizations in six countries. The aim is to develop a European pest risk analysis (PRA) for American and European populations of *P. ramorum*. A project objective is to disseminate results through outreach activities, formal presentations and the project website (http://rapra.csl.gov.uk). The website hosts an extensive database which has gathered together all the records of plants infected by *P. ramorum* in EU member states, as well as information on host susceptibility and differences between American and European populations of the pathogen.

During surveys to establish the extent of *P. ramorum* in the U.K., another new species of *Phytophthora* was discovered and has since been named *Phytophthora kernoviae*. Like *P. ramorum*, *P. kernoviae* is a serious foliar pathogen on rhododendron and also causes bleeding stem lesions on European beech (*Fagus sylvatica*). The current distribution of these two pathogens is described as well as the number of trees affected in Europe. In addition, some of the most recent research findings that have emerged from the RAPRA project are discussed including data on pathogen biology, breeding system and disease epidemiology, and compared with our growing understanding of the behavior of *P. kernoviae.*

Key words: *Phytophthora kernoviae*, *P. ramorum*, sudden oak death, rhododendron, bleeding canker, European ecosystems.

Introduction

Phytophthora ramorum (the cause of sudden oak death in California and Oregon) is acknowledged as a potential threat to European trees, woodland ecosystems and other environmentally important habitats. Only formally described and named in 2001 (Werres and others 2001), *P. ramorum* is recognized as a recent alien introduction to both the U.S. and Europe[3]. It is currently considered to be one of the most significant quarantine pathogens for Europe. Prior to being named, the pathogen was first

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[3] Commission decision of 19 September 2002 to prevent the introduction into and spread within the Community of *Phytophthora ramorum* Werres, De Cock & Man in't Veld sp. nov. (notified under document number C–2002 3380).

observed infecting foliage of rhododendron in Germany in 1993, but its relevance as the cause of sudden oak death (SOD) in the U.S. was not understood until 2001. However, the potential risk that SOD poses to Europe resulted in the European Commission decision of 19 September 2002. This put measures in place to stop further introductions of *P. ramorum* and prevent it spreading within Member States (MS). It included the requirement for all MS to carry out annual surveys of nurseries (and a year later also to survey the natural environment), to establish the status of *P. ramorum* in each country. In addition the European Commission funded the 3 year research project - Risk Analysis of *Phytophthora ramorum* (acronym RAPRA, FP6 Project 502672) to generate information about *P. ramorum* that was relevant to Europe and could inform changes to EU policy related to the pathogen.

Phytophthora ramorum in Europe

Since finding *P. ramorum* in southern England in 2002 (Lane and others 2003), the U.K. has reported around 580 outbreaks in nurseries/plant retail outlets, with more in gardens, parks and woodlands (http://www.defra.gov.uk/planth/pramorum.htm). Elsewhere in Europe *P. ramorum* has been found in nurseries and formally reported from 15 other countries: Belgium, Czech Republic, Denmark, Finland, France, Germany, Ireland, Italy, the Netherlands, Norway, Poland, Slovenia, Spain, Sweden, Switzerland, (although it is now considered as eradicated from the Czech Republic). As with the U.K., hosts of *P. ramorum* have mainly been ornamental plants from the genera *Rhododendron*, *Viburnum*, *Camellia* and *Pieri*s.

The findings of *P. ramorum* outside nurseries have included public and privately owned green areas as well as woodlands. In the U.K. (mainly England and Wales with just two outbreaks in Scotland) the disease has been confirmed at around 160 locations (http://www.defra.gov.uk/planth/pramorum.htm). Elsewhere in Europe (the Netherlands, Germany, Ireland, Norway, Slovenia and Switzerland) the number of findings of *P. ramorum* from outdoor locations varies widely but is generally much lower than the U.K., and the majority of infestations have been on rhododendron (see RAPRA website for more information: http://rapra.csl.gov.uk). In just two of these countries (England and the Netherlands), *P. ramorum* has also been found causing bleeding stem lesions on a range of tree species (table 1 and Brasier and others 2004).

In addition, it has become apparent in the infestations that occur beyond the confines of nurseries, both ornamental shrubs and some tree species can suffer from leaf and shoot infections. In the U.K. the evergreen oak, *Quercus ilex*, has been the most frequently found tree with *P. ramorum* leaf and shoot infections. However, other woodland species such as *Castanea sativa*, *Fraxinus excelsior* and *Q. cerris* as well as ornamental species of *Drimys*, *Magnolia*, *Michelia*, *Cinnamomum* and even *Eucalyptus* have been found with foliar infections (see http://rapra.csl.gov.uk). When this occurs, the disease has usually become well established on rhododendron prior to

Table 1—Trees with *P. ramorum* infected stem (bole) cankers in Europe.

Country	Tree Species*	Family	Number
England	*Aesculus hippocastanum*	Hippocastanaeae	1
England	*Acer pseudoplatanus*	Aceraceae	1
England	*Castanea sativa*	Fagaceae	1
England	*Fagus sylvatica*	Fagaceae	6
Netherlands	*Fagus sylvatica*	Fagaceae	6
England	*Nothofagus obliqua*	Fagaceae	2
England	*Quercus acuta*	Fagaceae	1
England	*Quercus cerris*	Fagaceae	5
England	*Quercus falcata*	Fagaceae	1
England	*Quercus petraea*	Fagaceae	1
Netherlands	*Quercus rubra*	Fagaceae	8
England	*Schima* sp.	Theaceae	1
Total			34

* Data from RAPRA database on naturally infected hosts, http://rapra.csl.gov.uk

foliage of trees becoming infected. Thus it is uncertain whether foliar infection of a European tree such as *Q. ilex* could sustain a *P. ramorum* epidemic in the same way that tanoak or bay laurel do in the U.S. Nonetheless, it remains a possibility and highlights the risk that this pathogen could pose to the Mediterranean holm oak forests of southern Europe (Moralejo and others 2006).

Factors Affecting *P. ramorum* Disease in Europe

In the U.K., *P. ramorum* has been found most often in planted woodland-gardens which host a wide range of non-native and exotic plants, but particularly where species and cultivars of rhododendron dominate. Where it is found infecting trees in woodlands, invariably a key understorey component is *Rhododendron ponticum*, an invasive species in its own right, which has become naturalized in many areas with acidic soils. Laboratory tests have shown that not only is rhododendron generally highly susceptible to infection by *P. ramorum*, but it can also support abundant sporulation (Denman and others 2006a, b; Morelajo and others 2007). There is no doubt that this host has played a key role in disease escape into the natural and semi-natural environments and the subsequent spread to trees. It is also the most frequently infected host in non-nursery outbreaks in other European countries.

However, in ecosystems in Europe where rhododendron is less abundant or absent, other plant species may take on the equivalent role and support abundant sporulation by *P. ramorum*. Some of the most important ecosystems at risk probably include the holm oak forest and laurisilva ('laurel forest') of southern Europe. These are home to several other tree and understorey species such as *Q. ilex*, *Rhamnus alaternus*, *Viburnum tinus,* and *Arbutus unedo,* all of which have the potential to support moderate to high levels of sporulation (Morelejo and others 2006). Species of *Vaccinium* have also been shown to be capable of supporting levels of sporulation

similar to those observed on Californian bay laurel and therefore could potentially sustain the disease in heathland habitats (Defra Report 2005).

Apart from the combination of trees with susceptible stems growing close to foliar hosts infected by *P. ramorum* and able to support abundant sporulation, climate plays a crucial role in disease establishment and development. To determine which parts of Europe could be at greatest risk based on climate alone, comparisons were made in 2003 using a climate matching model (CLIMEX) to compare southern Oregon, where *P. ramorum* is present, with Europe (R. Baker, Central Science Laboratory; member of the RAPRA Consortium). The comparison was then revised based on Meentemeyer and others (2004) and this identified the west of the U.K., Ireland and northwest parts of France, Spain and Portugal as regions with the closest eco-climate matching in relation to *P. ramorum*. Since then, the findings of *P. ramorum* outside nurseries have been most common in southwestern parts of England and Wales, where the combination of abundant understorey rhododendron and mild, often wet climate, appears to have provided a near perfect environment for the pathogen.

Another Aerial *Phytophthora*, *P. kernoviae*

Following the initial findings of *P. ramorum* in nurseries in the U.K., there have been a number of surveys; initially to determine how widely it was present and later to monitor spread. Identification of the areas most likely to harbour *P. ramorum*, based on climatic and host criteria, meant that particular emphasis was placed on surveying woodlands and woodland-gardens in the southwest of England. These revealed that a number of sites in Cornwall had *R. ponticum* and other *Rhododendron* cultivars heavily infected with *P. ramorum*, but also found another new and invasive *Phytophthora* species now named *P. kernoviae* (Brasier and others 2005). In much the same way as *P. ramorum*, *P. kernoviae* appeared to be a serious foliar pathogen on rhododendron and caused bleeding lesions on European beech (*Fagus sylvatica*).

Since the discovery of this new pathogen in November 2003 (Brasier and others 2004), *P. kernoviae* has been found extensively in an area of about 14.24 square km (5.5 square miles) in southern Cornwall between Redruth and Falmouth which is now defined as the *Phytophthora kernoviae* Management Zone or PkMZ (Anonymous 2004). It has been reported from one nursery in Cornwall, and also found at several other sites (mainly woodland) outside the PkMZ but still in the same county. There has also been a small cluster of outbreaks in Wales near Swansea, with a possible link between this cluster and one of the outbreak sites in the PkMZ. The only other occurrences of *P. kernoviae* in the U.K. have been limited to a single mature infected rhododendron in a managed garden and an outbreak in a nursery, both in the north of England and both now eradicated (Sansford 2007). Although *P. kernoviae* is considered to be one of several invasive tree *Phytophthoras* recently arrived in the U.K. (Brasier and others 2005), its origin is unknown and until recently it had not been reported from elsewhere. However, in March 2006 the New Zealand Ministry of Agriculture and Forestry announced findings of *P. kernoviae* in two separate localities on the North Island in New Zealand. At one of the sites it had caused necrosis on the shoots and fruits of cherimoya (*Annona cherimola*), and at the other site in the Trounson Kauri Park it had been isolated from soil. The status of *P. kernoviae* in New Zealand, either as a native or introduced species, has yet to be clarified.

Various aspects of the biology and epidemiology of both *P. ramorum* and *P. kernoviae* have been studied and compared. Both are aerial *Phytophthoras* which infect and sporulate abundantly on rhododendron, and both produce caducous sporangia. The two species apparently thrive under the same climatic conditions and can cause lethal bleeding cankers on mature trees and even overlap at the same site. This can lead to very intimate contact between the pathogens, and they have been found infecting the same rhododendron plant and even both causing separate bleeding cankers on the same tree (Brown and others 2006).

So far the range of tree species that *P. kernoviae* has been found infecting is much smaller than has been recorded for *P. ramorum.* The majority of trees with cankers caused by *P. kernoviae* consist of beech (around 55 in total), with two oaks (*Q. robur*) and one *Liriodendron tulipifera* infected. Lesion extension has been found to be very rapid in some trees, and can result in tree mortality in just a few years. As with *P. ramorum*, *P. kernoviae* infects the foliage of some ornamental shrubs (Beales and others 2006) but the host range is much smaller (primarily *Rhododendron* and *Pieris*). Some tree species have also been found with infected foliage; this includes a wide range of *Magnolia* species (Denman and others 2005), *Q. ilex*, *Michelia doltsopa* and *Drimys winterii* (S. Denman, unpublished data). There are also several critical differences between the two *Phytophthoras*. Not least, the two pathogens have different breeding systems: *P. ramorum* is heterothallic (Werres and others 2001) whereas *P. kernoviae* is homothallic (Brasier and others 2005). To date *P. kernoviae* has only been found in two nurseries in England, whereas *P. ramorum* has been found in close to 600 nurseries and garden centers throughout the U.K.

New Research Findings

Research has now been underway on the biology and epidemiology of both these *Phytophthora* pathogens for at least 4 years in Europe via RAPRA and other projects. The findings feed into the PRAs that are being undertaken to assess the risk that these pathogens pose to the U.K. and elsewhere in Europe. The outcome of work testing susceptibility to *P. ramorum* on more than 260 species and reports of 140 naturally infected host species in Europe are given in two large databases on the RAPRA website (http://rapra.csl.gov.uk). Even though susceptibility tests often introduce inoculum via wounds, it has been shown that zoospores of *P. ramorum* can penetrate mature, intact bark of tree species such as beech (*F. sylvatica*), sweet chestnut (*C. sativa*) and Douglas fir (*Pseudotsugae menziesii*) leading to infection (Brasier and Kirk unpublished). *Phytophthora ramorum and P. kernoviae* have also been recovered from discolored xylem 5 to 25 mm below exposed sapwood surfaces of naturally infected trees more than two years after the overlying bark was removed. Both pathogens probably commonly occupy xylem beneath phloem lesions and persist and even grow in that tissue (Brown and Brasier 2007). There is also evidence of the development of fungicide resistance in *P. ramorum.* In one study, around 25 percent of a sample of 71 *P. ramorum* isolates obtained mainly from infected nursery plants from various European countries showed resistance to the chemical metalaxyl-M (Wagner and others 2004).

Understanding the breeding system of *P. ramorum* and the potential for interbreeding between European and American lineages of *P. ramorum* (Ivors and others 2006) remains central to assessing the risk this pathogen poses to Europe and indeed other parts of the world. As a heterothallic species, both A1 and A2 mating types are required for sexual recombination in *P. ramorum* and the European lineage is

predominantly A1 whilst in the U.S. it is A2. Extensive testing of isolates from Europe and America as part of the RAPRA project has confirmed this separation between continents of the mating types with two exceptions (Werres and others 2007). These are 1) findings of A1 European lineage in nurseries in Oregon and Washington state (Hansen and others 2003) and 2) the discovery of at least three isolates in Belgium which appear to be of European lineage but of A2 mating type (minutes of the EU Plant Health Standing Committee meeting on 26–27 November 2006). Moreover, in studies which looked at the success of mating between A1 and A2 isolates of *P. ramorum*, a surprisingly high frequency (average about 57 percent) of gametangia were abnormally developed or contained visibly aborted oospores; an even higher proportion were classified as non-viable following vital staining (Brasier and others 2007). Despite this, although the frequency of viable, mature oospores in *P. ramorum* is comparatively low (compared with other *Phytophthora* species), there is clearly potential for genetic recombination of *P. ramorum* via the sexual stage.

However, probably some of the most striking, recent findings about both *P. ramorum* and *P. kernoviae* relate to the discovery of asymptomatic aerial infection following exposure in the field to these pathogens. Furthermore, there is evidence of sporulation from some of these asymptomatic leaves and fruits (Denman and others 2007). Infected but symptom free roots may also harbour *P. ramorum*. Following exposure to zoospores and a period of incubation, potted *Rhododendron* plants were examined using microscopy and revealed there were *P. ramorum* chlamydospores visible in the roots but no evidence of necrosis (Reidel and others 2007). Both findings have clear implications for the control of disease spread by these pathogens, particularly for the nursery trade.

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Determining the Effectiveness of the Federal Order/Interim Rule on *Phytophthora ramorum* Dissemination in Nurseries[1]

Karen Suslow[2]

Abstract

When we examine the nursery survey data over the past 3 years, we find that in the western states, the number of *Phytophthora ramorum*-infested nurseries found via nursery inspections or surveys has dropped by more than 50 percent from 2004 to 2006, from 110 nurseries to 50, respectively. The percent of nurseries found to be infested compared to the number of nurseries inspected was at 1 to 2 percent. There were three main factors that were responsible for, or influenced, the decline from 2004 to 2006:

1. The sudden oak death Federal Order which was signed in 2004 and implemented in January 2005.
2. Grower trainings which were conducted beginning in January through March, 2004, sponsored by the California Oak Mortality Task Force (COMTF) and
3. Critical nursery research that was being identified and conducted.

In order to continue the trend, the United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA APHIS), National Plant Board (NPB) and the nursery industry are working together on advanced training for growers, additional inspections of high-risk plants and piloting a voluntary best management practices (BMPs) program in the three western states in 2007. Thus as the regulations are pulled back, the BMPs may serve as a stepping stone to a clean stock-like program for all interstate shippers of nursery stock in the United States.

Key words: *Phytophthora ramorum*, sudden oak death.

When I was asked to give a talk at the Sudden Oak Death Third Science Symposium, I looked back at my previous talks and noted that I have spoken at length on the challenges that the industry and regulators face when working with a newly introduced pest. In reviewing the past 5 years, I would like to focus on how effective the majority of the regulations have been at minimizing the dissemination of the pathogen on nursery stock shipped interstate.

When we examine the inspection and survey data over the past 3 years, we find that in the western states, the number of infested nurseries found via nursery inspections or surveys has dropped by more than 50 percent from 2004 to 2006, from 110 nurseries to 50, respectively.

The percent of nurseries found to be infected compared to the number of nurseries inspected was at 1 to 2 percent.

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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Corresponding author: K. Suslow, ksuslow@hineshort.com.

There were three main factors that were responsible for, or influenced, the decline from 2004 to 2006:

1. The sudden oak death Federal Order which was implemented in January 2005.
2. Grower trainings which were conducted beginning in January through March, 2004, sponsored by the COMTF.
3. Critical nursery research that was being identified and conducted.

The introduction of the Interim Rule led to the annual nursery inspections with a focus on *Phytophthora ramorum*-like symptoms and buy-in restrictions on nurseries that shipped host and associated host plants (HAP) interstate. Interstate shippers of HAP, if purchasing plants from other vendors, were required to purchase the product from nurseries that also possessed a USDA sudden oak death (SOD) Compliance Agreement or appropriate phytosanitary certification.

However, prior to the introduction of the Federal Order in January of 2005, buy-in pathways from Europe and British Columbia were not monitored in nurseries from other parts of the country. The front door was guarded in that nurseries in the rest of the U.S. put restrictions on product coming from the western states, but they failed to block the back door which was open to trade from potentially infected countries. My feeling is that the same situation existed for nurseries in the west coast prior to the establishment of the federal Emergency Order.

I feel that finds in nurseries from 2003 to 2006 are reflective of unrestricted trade in the late 90s and early 2000 between potentially infested states and between potentially infested countries. The pathogen entered the trade on the west coast, whether via nursery stock or the native environment, the inoculum built up in nurseries and it was not until 2003 to 2004 that the first noticeable finds occurred in nurseries shipping interstate.

Secondly, in addition to the Interim Rule, growers were made aware of the symptoms of the disease on ornamental plants, where the disease was found in nature, and, through grower trainings, were engaged in a broader scale discussion and review of BMPs for the prevention of the introduction of *P. ramorum* into a nursery operation.

Lastly, the nursery industry had identified needed research which would enable policies to be based on sound science (this continues to be work in progress).

We discussed the factors that influenced or contributed to the trend from 2004 to 2006, but what additional factors can we implement that will continue to influence this trend going forward? Let’s analyze the data and determine what has been effective in reducing the occurrence of the pathogen in nursery trade. In quarantined counties, it has been suggested that the monthly inspections of interstate shippers of HAP has prevented potentially infected material from being shipped out of state. Conducting the inspections during the time of year when the disease is most prevalent (during the transition period from fall to winter and from winter to spring) has been shown to be far more successful at finding the disease on ornamental crops than inspections during off times.

The data also indicates that approximately 90 percent of the time, if there is an infection, a high risk (HR) plant, camellias and rhododendrons, is the ornamental plant found infected or associated with another infected plant.

So with that in mind, APHIS, the NPB and nursery industry discussed and identified that additional inspections are needed of HR plants that are shipped interstate and, if funding is available, for medium risk genera, such as *Pieris, Viburnum,* and *Kalmia*. Additionally, monthly inspections of HR plants by trained nursery personnel were proposed. COMTF, along with The California Department of Forestry (CDFA), The United States Department of Agriculture-Forest Service (USDA-FS) and the nursery industry in California will conduct trainings for growers up and down the state, similar to the trainings offered in 2004. Oregon is also formulating a training plan.

With regards to recurrent or repeat nurseries… those that have been found to be infected a second or third year likely due to their soil or water being infested, these nurseries need the attention of researchers to identify an effective method of soil/water remediation. There will be several talks during this conference focusing on soil cleanup.

Finally, as we roll into 2007, we need to focus on risk mitigation measures to prevent the introduction of *P. ramorum*, as well as other pests and pathogens, into nursery operations. The BMPs or risk mitigation measures were designed by researchers, regulators, and industry for the sole purpose of assisting nursery growers in identifying areas of risk in their operations. Preliminary discussions have been occurring with regards to a pilot program of the BMPs in the western states. The objective would be to create a pilot program for 2007, learn what is and is not successful, so that by the time the Emergency Order sunsets, we will have a viable program in place, which could potentially be the BMPs. The BMPs could then be a stepping stone to a clean stock-like program in the future.

The criteria for the pilot program may be as follows:

- Be conducted in the western states; include small, medium and large facilities in both quarantined and regulated counties.
- The nursery participating in the pilot program would partner with the state or county agriculture department and determine the BMPs that are appropriate for a particular nursery based on a wide range of factors, including the physical location of the nursery - whether it is located in a quarantined or regulated county, geographical location – hilly terrain, streams on property, environmental factors, crops grown and type of nursery – in-ground, containerized, greenhouse. Auditing of the program could occur annually or whenever the nursery modified their operations.

How could auditing of voluntary BMPs be accomplished? First, the following criteria would need to be identified before the appropriate BMPs could be suggested. A number of nurseries have already gone through the process of identifying needed risk mitigation measures to prevent the introduction of pathogens into their operations. The BMP pilot program would be documenting what the nursery currently has in place, ensuring that the program contains all the components of the BMP program and auditing it.

Secondly, a customized workbook could be created for each pilot site. Currently draft standardized documentation sheets for each BMP are being created which identify how a practice is being addressed. If the BMP is deemed appropriate for the site, then the 'site specific nursery' check-off box would be marked. If the practice were not appropriate, the 'N/A' box would be checked. The draft BMP documentation sheets will be vetted to the industry and regulators. These would create the framework for the workbook.

So for example, if this recommended BMP were selected as appropriate for a nursery, "Avoid or minimize accumulation of standing surface water in containerized high-risk plant beds" then the following documentation of irrigation practices would be completed. The options to check off are:

- Sufficient drainage available to prevent standing water in high risk production areas.
- High risk plants are grown on benches which allow for drainage.
- Gravel or other highly permeable surface under HR plants allows for water drainage sufficient to prevent standing water.
- Slope of land drains water away from HR production areas.

Another example of a recommended BMP with documentation is:
"Avoid overhead irrigation of high-risk plants. Irrigate in a manner to avoid prolonged leaf wetness of 12 hours or more." The options are:

- Overhead irrigation is not used on HR plants.
- Timing of overhead irrigation is early enough to allow for leaf drying.
- Circulation fans are used.
- Plant spacing is adequate and allows for foliage to dry within 12 hours.
- Other methods are used to minimize leaf wetness (explain).

In addition, Tuesday night the California Oak Mortality Task Force nursery committee, which is open to everyone, will meet to review and update the most current nursery research needs list. This list will be the basis for research that will be conducted at the W501 mock nursery site in California. Talks are ongoing in Alameda County with regards to a 7.28 ha (18 acre) parcel, five of which we are interested in setting up as a mock nursery. Kathy Kosta and Nik Grünwald will be giving an update at the nursery committee meeting.

We hope to have a diagnostics firm working with the researchers at the mock nursery to design simple, fast, accurate, field diagnostics for the detection of *P. ramorum* infected plants.

So as we move into 2007 and beyond, we expect the positive trend in the reduction of infested nurseries to continue as we utilize the BMPs as a potential stepping stone towards a long-range goal of a 'clean stock-like program' for all interstate shippers of nursery stock in the U.S.

An Update on *Phytophthora ramorum* in European Nurseries[1]

David Slawson,[2] Jennie Blackburn,[2] and Lynne Bennett[2]

Abstract

Emergency phytosanitary measures to prevent the introduction into and spread within the European Union (EU) of *Phytophthora ramorum* Werres, De Cock & Man in 't Veld. have been in place since 2002. Surveillance across the EU, has confirmed the presence of *P. ramorum* on nurseries and retailers in 15 member states. *Phytophthora ramorum* has been confirmed on 14 plant genera at nurseries and retailers, with 96 percent findings being made on *Rhododendron*, *Viburnum* and *Camellia*, which are the three genera currently regulated for internal movement within the EU. More detailed analysis of data from England and Wales shows that rigorous enforcement of the emergency measures can result in very encouraging reductions in compliance infringement both in terms of documentary errors and findings of *P. ramorum* on certificated material. Furthermore, the percentage of inspections positive for *P. ramorum* and the number of outbreaks at nurseries and retailers has shown similar and encouraging reductions between 2002 and 2006. *P. ramorum* has, however, continued to be found in the EU on commercially-traded plants, which indicates that the emergency phytosanitary measures have not been completely effective. Any future revisions to the measures need to take account not only of emerging results from on-going research and surveillance but also of compliance costs to official services and to the commercial nursery stock industry in relation to benefits.

Keywords: *Phytophthora ramorum*, sudden oak death, European Union, emergency phytosanitary measures.

Introduction

Emergency phytosanitary measures to counter the threat posed to the European Union (EU) from *Phytophthora ramorum* were introduced in September 2002 (Anonymous 2002). These measures were amended in April 2004 (Anonymous 2004 a, b) and again in March 2007 (Anonymous 2007). In summary, the current measures require member states of the EU to conduct official surveys, to apply import controls and internal movement controls on *Rhododendron* spp. L., *Viburnum* spp. L. and *Camellia* spp. L. and to require that eradication measures are taken against findings of *P. ramorum* at places of production. Nurseries are subject to at least two official inspection visits to confirm place of production freedom, and any findings of *P. ramorum* require further eradication measures. The latest revised eradication measures consist of the destruction of infected plants, susceptible plants in a 2 m radius of infected plants and destruction of associated growing media. Appropriate hygiene measures must also be applied to the standing surface. Movement controls are also required on all susceptible plants in a 10 m radius of infected plants and on any remaining plants in the affected lot. These plants must be held for at least 3 months, during which they must not be treated with any anti-*Phytophthora*

[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9 2007, Santa Rosa, California.

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fungicides, and they must be visually inspected at least twice to confirm freedom from *P. ramorum*. All other susceptible plants on the premises must also be subject to official inspection. Consignments of *Rhododendron*, *Viburnum* and *Camellia* moving within the EU must be accompanied by a "plant passport", which is in effect an official document confirming that the plants comply with required official measures.

This paper provides an initial analysis of the effect of these measures on the prevalence of *P. ramorum* during the period 2004 to 2006.

Results

European Union

Survey results of nurseries and retail premises for the years 2004, 2005 and 2006 show that *P. ramorum* has been found on plant species in the following genera: *Rhododendron*, *Viburnum*, *Camellia*, *Pieris*, *Kalmia*, *Leucothoe*, *Magnolia*, *Osmanthus*, *Laurus*, *Salix*, *Taxus*, *Arbutus*, *Hamamelis,* and *Syringa*. The incidence of findings on individual genera is not available for the EU but data from England and Wales for the same period show that 96 percent of findings of *P. ramorum* at nurseries and retail premises have been on *Rhododendron* (47 percent), *Viburnum* (41 percent) and *Camellia* (8 percent). Results also show that *P. ramorum* is present in the following EU countries: Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Ireland, Italy, the Netherlands, Poland, Slovenia, Spain, Sweden, and the United Kingdom. The results show that a fairly consistent survey regime, in terms of visual inspections and laboratory testing of samples, has been maintained across the EU (fig. 1), and encouragingly there has been a steady reduction in the number of new outbreaks on nurseries and retailers from 255 in 2004, to 203 in 2005 and to 108 in 2006.

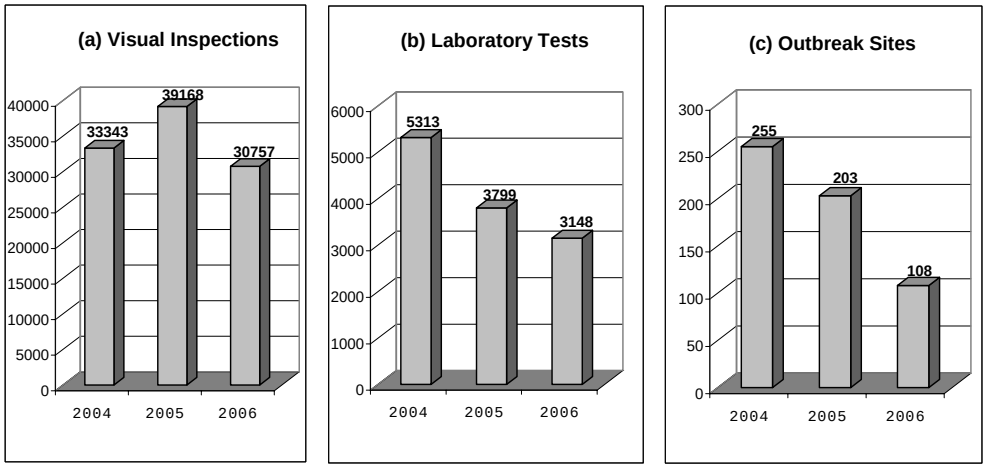

Figure 1–EU Nursery and retailer survey results 2004-2006. Number of (a) Visual Inspections, (b) Laboratory Tests and (c) Outbreak Sites.

England and Wales

More detailed data are available for England and Wales. Over the period 2003 to 2006, the incidence of documentary infringements (where an infringement represents either the absence of a "plant passport" or an incorrect "plant passport") fell from 109 in 2003 to 5 in 2006, a reduction of 95 percent (fig. 2); findings of *P. ramorum* on

"plant passported" consignments fell from 128 in 2003 to 20 in 2006, a reduction of 84 percent (fig. 3), and the percentage of inspections that were positive for *P. ramorum* fell from 2.7 percent in 2003 to 0.8 percent in 2006 (fig. 4). The number of outbreaks of *P. ramorum* at nurseries and retailers in England and Wales has also reduced over the period, from a peak of 161 in 2003 to 34 in 2006 (fig. 5).

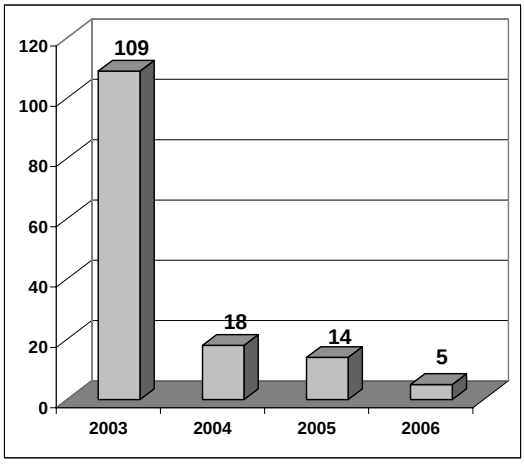

Figure 2–Number of Documentary Infringements in England and Wales (2003-2006).

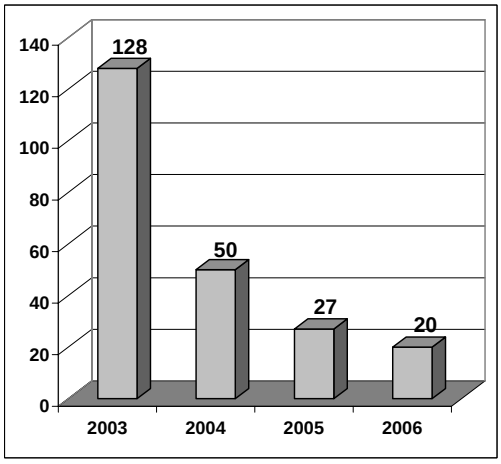

Figure 3–Number of findings of *Phytophthora ramorum* on "Plant Passported" consignments in England and Wales (2003-2006).

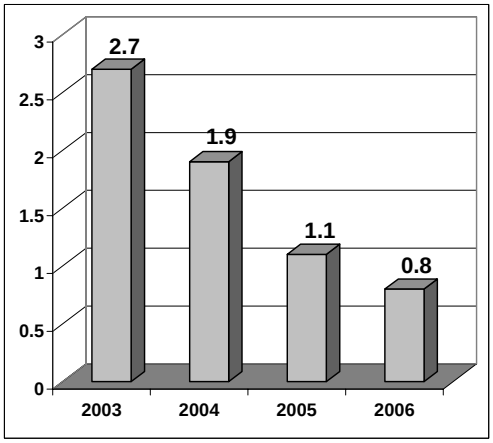

Figure 4–Percentage of inspections positive for *Phytophthora ramorum* in England and Wales (2003-2006).

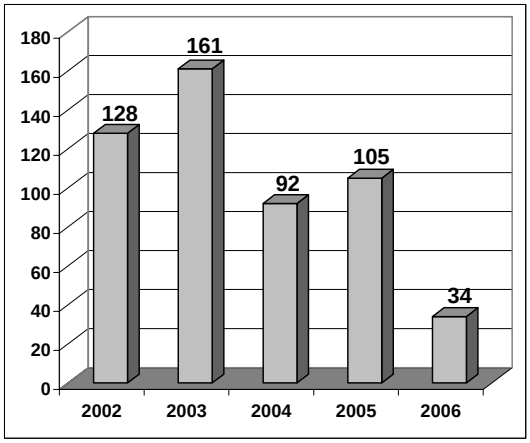

Figure 5–Number of outbreaks at nurseries and retailers in England and Wales (2002-2006).

Discussion

The tentative conclusion of this trend analysis is that the required official measures can, if rigorously applied as they have been in the England and Wales, reduce very significantly the incidence of *P. ramorum* moving on commercially traded plants. The measures, however, have not been completely effective, and *P. ramorum* has continued to be found at nurseries and retailers in many countries of the EU. It is hoped that results of on-going research and surveillance will enable the measures to

be refined in order to increase their effectiveness. Some support to the importance of continued action on places of production is provided by results emerging from epidemiological modelling studies on *P. ramorum*. These results indicate a "scale-free" network, for which it is postulated that action at the critical "nodes" (in other words, the places of production) will be highly effective at limiting future spread (Jeger and others 2007). Any future revision must also take account of the high costs of compliance to official services and to the commercial nursery stock industry in relation to benefits to the trade and to the environment.

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Canadian *Phytophthora ramorum* 2006 Update[1]

Ken Wong[2]

Abstract

Annual national surveys for *Phytophthora ramorum* have been conducted in Canada since 2002. Over 37,361 samples were taken in 2006, which focused on 251 wholesale and retail nurseries across Canada that were either growers or importers of host plants. In 2006, the national survey detected *P. ramorum* at one wholesale and four retail nurseries in the province of British Columbia. To-date in Canada, there has only been regulatory incidents of *P. ramorum* at nursery businesses, restricted to areas of the Lower Mainland, Vancouver Island and Sunshine Coast of British Columbia. Trade in nursery plants with currently known infested states is thought to be the source of inoculum for these nurseries. The Confirmed Nursery Protocol or Enhanced Confirmed Nursery Protocol has been implemented to eradicate *P. ramorum* at these sites.

The Confirmed Nursery Protocol, employed at the four retail nurseries, involves: the quarantining of all *P. ramorum* hosts until the completion of a delimitation survey; destruction of infected block(s) of host plants; destruction of cull pile(s); sampling of water and soil; as well as a ninety day quarantine of host plants that are found within ten meters of destruction block(s).

An Enhanced Confirmed Nursery Protocol was developed for the repeat wholesale nursery. A number of factors unique to this wholesale nursery are thought to have contributed to the severity of the situation, such as crowding of plants, overhead watering in greenhouses and flooding. This protocol was developed to increase the success of eradication and required: restricted plant movement at the nursery; intensive survey and sampling of all plant genera; the quarantining of all plants within ten meters of the destruction block(s); and increased soil sampling and water baiting. A total of 8,772 plant samples, 185 soil samples, and 43 water samples, were taken at this wholesale nursery. Five soil positive areas and the following five new host genera were found as a result of this protocol: *Distylium myricoides* Hemsl., *Manglietia insignis* Blume, *Parakmeria lotungensis* (Chun & C.H.Tsoong) Y.W.Law, *Ilex purpurea* Hassk., and *Loropetalum chinense* Oliver. Plant destruction was accomplished by deep burial or burning, while soils were either fumigated or covered with concrete. To further ensure success, mandatory management practices were implemented such as: restrictions on the movement of plants, soil, staff,

[1] A version of this paper was presented at the Sudden Oak Death Science Third Symposium, March 5–9, 2007, Santa Rosa, California

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equipment; recording of all plant locations; training of staff; a visitor policy; plant debris management and sanitation requirements.

Eradication efforts for *P. ramorum* in Canada however, come at a cost and necessitate a constant assessment of the regulatory approach. Canadian regulators will need to keep in mind questions the questions listed below.

What are we learning from science?
Is the risk to Canada lower than once feared?
Has uncertainty been reduced?
Do we need a more aggressive approach for repeat nurseries?
Would a certification program for high risk host plants help?
How are trading partners viewing the situation?
From a risk management perspective, where to draw the line?

Key words: *Phytophthora ramorum*, British Columbia, Confirmed Nursery Protocol, Enhanced Confirmed Nursery Protocol, new host plants.

Phytophthora ramorum and *P. kernoviae*: Regulation in the European Union[1]

Stephen Hunter[2]

Abstract

The history of the regulation of action against *Phytophthora ramorum* and *P. kernoviae* in the EU and U.K. is briefly summarised. For the former there are EU controls on the import of host plants, and the internal regime of plant passporting has been extended to cover *Rhododendron*, *Viburnum* and *Camellia*. There are also requirements relating to containment and eradication at infected nurseries. For *P. kernoviae* the U.K. has taken action through national legislation which includes the introduction of a management zone in the most heavily infected area of Cornwall. Some pilot work on the clearance of *Rhododendron* has also occurred. In the U.K. funding for the *Phytophthora* Programme will run out during 2007/08 and a scientific and policy review will take place in order to set the direction of long term policy for both pathogens. This will involve a public consultation later in 2007. Future policy actions are likely to be discussed in the EU Plant Health Standing Committee in 2008.

Key words: *Phytophthora ramorum*, *Phytophthora kernoviae*, European Union, regulation.

History of EU and U.K. Legislation

Phytophthora ramorum

Regulatory action against *Phytophthora ramorum* falls under Article 16 of the European Union Plant Health Directive. Member States must report new pests or pathogens and can take emergency action to contain or eradicate them. Such action has to be reported to the EU Commission which then considers them with experts from the Member States at the Plant Health Standing Committee, normally within three months. The Standing Committee may then adopt EU-wide emergency measures which supersede any national ones already introduced.

The first finding of *P. ramorum* in the U.K. was at a nursery in April 2002. The Government introduced emergency measures in May 2002 and these were replaced by an EU Decision in the following November. Subsequently the Decision has been amended twice, in April 2004 and February 2007.

The original measures placed controls on the importation of host species into the European Union from third countries. The established internal EU plant passporting regime was extended to include the two most common hosts *Rhododendron* and *Viburnum*. Containment and eradication measures were introduced at nurseries where infection was found. The first amendment in 2004 extended the list of host species covered and included *Camellia* in the plant passporting regime. Notification of suspect occurrence by producers was made mandatory, already a generic requirement

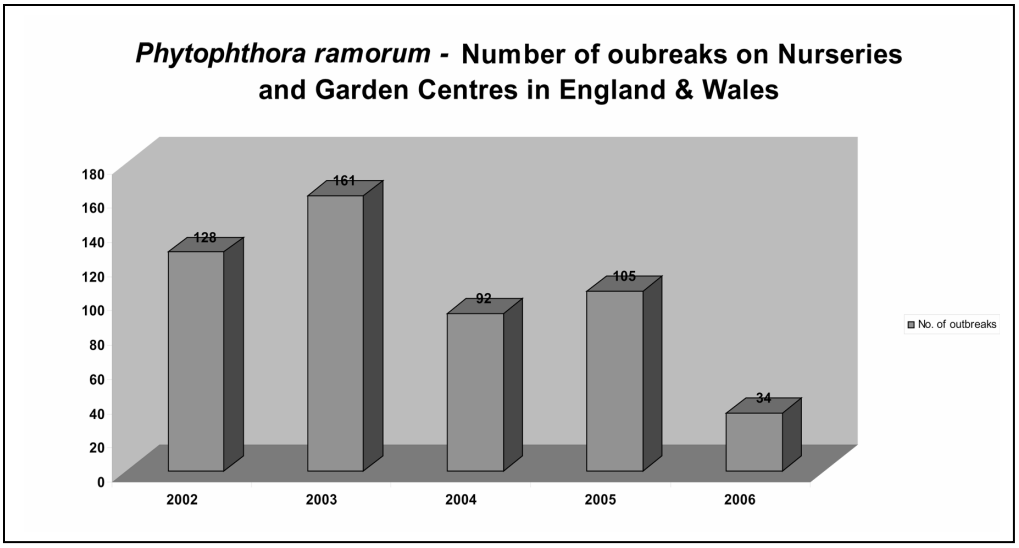
[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9 2007, Santa Rosa, California.

[2] Dr. Stephen Hunter, Deputy Director (Plant & Bee Health), Defra, stephen.hunter@defra.gsi.gov.uk.

under U.K. legislation. An obligation to take, at least, containment measures at non-nursery outbreaks was introduced. In 2007 further adjustments were made to the host list in light of international findings. Inspections of host plants at nurseries were increased to at least two per year. Further changes await the outcome of an EU-funded research project called RAPRA.

The number of outbreaks at nurseries and garden centres in England and Wales is shown in figure 1. In addition to fulfilling EU legislative requirements the U.K. introduced a regime of inspection which involved four visits annually to nurseries and an increased level of checks on material arriving from the rest of the EU.

The success in reducing the level of outbreaks in England and Wales from a peak of 161 in 2003 to 34 in 2006 will allow a reduction in nursery inspections to three and a lower level of checks on material of EU origin during 2007. However, this level of inspection is being kept under review.



Phytophthora kernoviae

A new species, *Phytophthora kernoviae*, was first found in the U.K. in 2004 during surveys for *P. ramorum* in Cornwall. Recently, New Zealand became the only other nation to find this pathogen. The U.K. introduced emergency measures in the same year. Surveys and inspections now routinely cover both species. There have been only two nursery outbreaks and eradication action has been successfully undertaken at both sites. The remaining outbreaks are non-nursery in nature and are predominantly in the county of Cornwall. Given the nature of the outbreaks in Cornwall the U.K. introduced legislation establishing a management zone for *P. kernoviae*. The movement of host material from the zone is prohibited except with the authority of an inspector and there is a power to close rights of way (footpaths with historical public access). The management zone approach allows the coverage of about 2,000 households without having to issue individual notices. Outbreaks outside the zone are dealt with as for *P. ramorum* through the issue of individual notices. In addition to these legislative measures some public money has been used to carry out *Rhododendron* clearance from high risk sites on a pilot basis.

The U.K. reported these measures to the EU Commission and Standing Committee. Other member states have been asked to test for both species during the annual *P. ramorum* survey.

Future Policy

U.K. Consultation

In the U.K. the situation appears to have stabilized with a continuing reduction in the number of outbreaks on nurseries and the majority of non-nursery outbreaks of both pathogens being located within the southwest of England. However, the additional government funding provided for the *Phytophthora* Programme will have been exhausted by March 2008, probably earlier. Before a future policy approach can be determined there will need to be a formal public consultation during the autumn of 2007. Unlike previous consultations this will present options for longer term policy rather than for emergency precautionary action. The existing funding was never designed to resource a full scale *Rhododendron* clearance programme in infested areas. The current pilot scale clearance activities will need to be evaluated and a cost-benefit analysis of such work undertaken.

The consultation will need to be based on agreed data and experience and on an analysis of the state of science regarding these two pathogens. There will be the results of the RAPRA project to be taken into account if they are available in time and a *P. ramorum* data sheet agreed by international scientists. The pest risk analysis (PRA) will have to be relevant to the U.K. and EU. Key questions for the U.K. will need to be answered separately for *P. ramorum* and *P. kernoviae*. From a policy perspective, we will need to know the extent of the current damage caused by these pathogens and what would happen without controls? What sort of mitigation might work? How do the risk pathways operate and who should bear the cost of any control activities or of the damage caused by the pathogens?

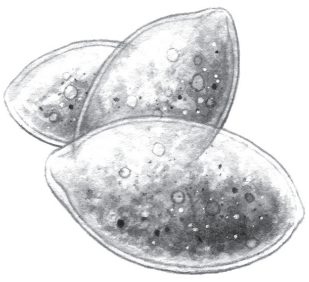
The format of the consultation will be agreed and managed by the U.K inter-departmental *Phytophthora* Programme Board who will be assisted by science and industry liaison sub-groups. The public consultation will last 12 weeks with the documents and background information placed on relevant government websites. There will also be a number of public meetings. The results of the consultation will be placed in Parliament and will inform future policy action and the U.K. negotiating position during any subsequent EU review.

EU Policy Review

There is likely to be a review of the amended EU Decision relating to *P. ramorum* and *P. kernoviae* during 2008. This will consider the technical detail of the Decision such as host lists, eradication requirements and whether any particular treatments (chemical or otherwise) should be banned, encouraged or mandated. However, more fundamental questions about whether action on non-nursery outbreaks should be left to the discretion of Member States and whether action on *P. kernoviae* should be left to U.K. discretion will also be asked. Ultimately, Member States will be asked whether the emergency measures should be made permanent or dropped.

The EU review will be undertaken by the Standing Committee and will be based on output by the RAPRA project. Member States may well hold a wide range of views. The Commission will attempt to gain a consensus but could call a vote in order to obtain a decision on the way forward.

Landscape Monitoring and Mapping



Phytophthora ramorum

Natural Outbreaks of *Phytophthora ramorum* in the U.K.—Current Status and Monitoring Update[1]

Judith Turner,[2] Philip Jennings,[2] Gilli Humphries,[2] Steve Parker,[2] Sam McDonough,[2] Jackie Stonehouse,[2] David Lockley, [3] and David Slawson[4]

Abstract

To date (February 2007) there have been 160 outbreaks of *Phytophthora ramorum* in gardens or woodlands in the U.K. Current EU policy requires that appropriate measures be taken to contain *P. ramorum* in such situations. In the U.K., the measures have either been aimed at eradication, through destruction of infected plants, or at containment to minimise the risk of *P. ramorum* being spread from the site to other areas. Of the 160 natural outbreaks recorded, 123 are ongoing, whilst the remainder are considered to have been eradicated as no further plant infections have been recorded, although in some cases the pathogen may still be detected as residual inoculum in soil or water. Monitoring of residual inoculum levels in soil/leaf debris has been carried out monthly for a period of up to three years in several sites in the south of England to investigate the extent of contamination within the gardens or woodlands and to quantify the effect of season on variation in inoculum levels. A number of the gardens were also found to be infected by *P. kernoviae* and in those situations the monitoring was extended to include both pathogens. A range of methods for monitoring levels of *P. ramorum* and *P. kernoviae* has been evaluated for routine use within the project. Initially samples were analysed using rhododendron leaf bait methods followed by isolation and identification on selective agar. All positive identifications were then confirmed using Real-Time Taqman PCR (polymerase chain reaction). More recently, PCR methods have replaced isolation and identification steps and have been used to develop more quantitative methodologies for monitoring seasonal changes in inoculum levels. Results have confirmed that the pathogens can survive and establish in the U.K. environment and that inoculum levels fluctuate in response to seasonal weather factors. During 2006, additional studies have been carried out to monitor inoculum movement from infected plants and has confirmed long distance (>50m) dispersal during wind-driven rain.

Key words: *Phytophthora ramorum*, sudden oak death, *Phytophthora kernoviae*, monitoring, eradication, dispersal.

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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Introduction

Outbreaks of *Phytophthora ramorum* in large managed gardens were first detected in the U.K. in 2003 and a research programme was commissioned by Department for Environment and Rural Affairs (Defra) to monitor residual contamination in affected areas following eradication action. The monitoring was aimed at investigating key questions to inform risk analyses for the pathogen in the U.K. These included seasonal pathogen survival, the levels of pathogen persistence in water, leaf litter and soil, and inoculum dispersal in the presence/absence of rainfall. Originally, the monitoring was focused on *P. ramorum* but was extended to include *P. kernoviae* when it was discovered in late 2003. The project has tested and validated a range of monitoring methods including the use of quantitative PCR for detection of very low levels of inoculum in soil and water.

Materials and Methods

Sites

Levels of residual inoculum remaining in soil and water following removal of diseased plants were monitored at a number of sites in southern England between 2003 and 2006. For the purposes of this paper, monitoring data are reported from three sites (A, B, C), which are representative of the findings from the wider monitoring programme. Site A was a large managed garden in the southeast, in which eradication action on all infected plants had been taken following an outbreak of *P. ramorum*. Site B was a large managed garden in southwest England, which was affected by outbreaks of both *P. ramorum* and *P. kernoviae* and where only localised eradication action had been taken. The third site (Site C), a large managed garden in the southwest affected by outbreaks of both *P. ramorum* and *P. kernoviae* and where very limited eradication action had been taken. At all sites the outbreaks occurred primarily on cultivated or wild rhododendron species.

Monitoring of Residual Inoculum in Soil and Water

Grids composed of 1 m x 1 m quadrats were marked out in selected areas where previously infected plants had either been removed (Site A) or about to be removed (Site B). Soil and leaf litter samples were taken from each quadrat at roughly monthly intervals and examined for the presence of *P. ramorum* and/or *P. kernoviae*. At site A, where an extensive watercourse was present, baits (rhododendron leaves contained in muslin bags) were deployed along streams, and in ponds, for a period of 1 to 3 days and then removed for testing using baiting methods. Monitoring was carried out at three-month intervals between 2004 and 2006.

Monitoring of Inoculum Dispersal During Rainfall

Two types of rain trap were used, one at ground level to collect splash-borne inoculum and a second attached to a pole at approximately 1 m above ground level to collect inoculum moving above ground during rainfall. The majority of the rain traps were sited near to or under infected plants and sampled every four weeks for presence of spores. However, a few traps were placed at distance from any infected host plants to monitor longer distance dispersal.

Diagnostic Methodologies

All samples were analysed for presence of *P. ramorum* and *P. kernoviae* by leaf baiting, isolation and microscopy methods, with any positives confirmed by Real-Time TaqMan PCR (Hughes and others 2006; Hughes, 2007, personal communication). In 2006, new DNA extraction protocols were validated for use in monitoring trace levels of *P. ramorum* or *P. kernoviae* in soil at Site A and rain water samples from traps at Site C. Firstly, calibration curves were determined using samples of soil or water to which known numbers of sporangia of *P. ramorum* or *P. kernoviae* had been added. DNA was then extracted from these samples and tested for the presence of the target pathogen DNA using TaqMan PCR. The resultant Ct values were plotted against the original number of spores added to each sample to examine and calibrate levels of detection. Results indicated a strong relationship between number of spores present and the Ct value using PCR.

Results and Discussion

Persistence of Inoculum in Soil (Site A)

Monitoring using traditional baiting methods, undertaken monthly between 2003 and 2005, indicated sparse but persistent levels of *P. ramorum*, with the number of positive grids fluctuating seasonally. Inoculum was more widespread between October and March compared with the summer months. In 2007, the site was revisited and samples taken for testing using both traditional and quantitative diagnostic tests. Despite consistently negative baiting results, quantitative PCR detected inoculum of *P. ramorum* at some sites but also demonstrated that inoculum was absent from some previously contaminated areas. The locations that were free from inoculum were either areas where no run-off was occurring from other parts of the gardens or where root material from the original infected plant had also been removed.

Persistence of Inoculum in Soil (Site B)

Monitoring at site B was initiated in December 2003, six months prior to the removal of the infected plants (between June and August 2004). Soil samples were taken monthly and the percentage of grids positive for either *P. ramorum* or *P. kernoviae* determined using baiting methods. Incidence of both pathogens was found to fluctuate seasonally with peaks in inoculum levels occurring between October and March (fig. 1). Incidence of *P. ramorum* has persisted since monitoring began whereas that of *P. kernoviae* has declined over time.

Evidence from both sites indicates that rapid and thorough action involving removal of all infected plants, litter, and preferably the root material as well, can be effective in reducing inoculum levels to below the current thresholds for detection using baiting and isolation methods. Although inoculum distribution fluctuated seasonally, reductions in inoculum presence over time have been demonstrated under these scenarios. A comparison of the data on *P. ramorum* and *P. kernoviae* indicated that *P. kernoviae* is less persistent in soil, possibly due to the fact that it does not produce chlamydospores (there is no evidence of the presence of oospores in the natural environment in the U.K.).

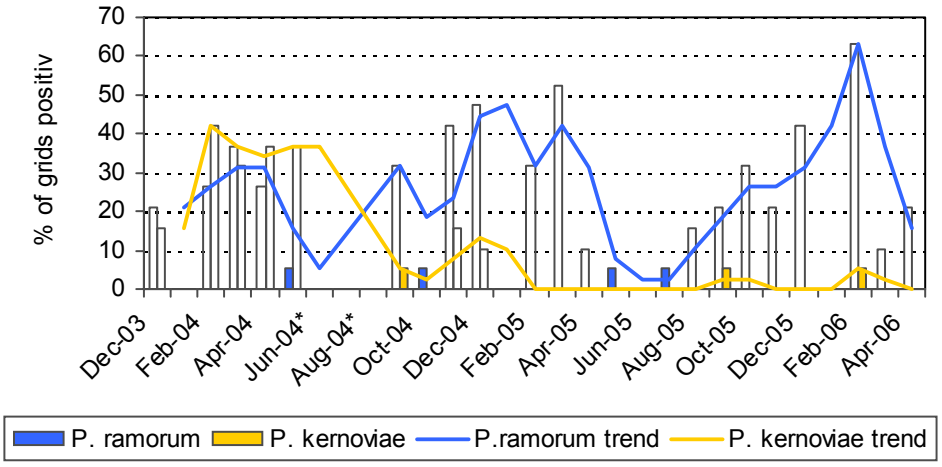

Figure 1—Detection of *P. ramorum* and *P. kernoviae* in soil between December 2003 and April 2006 at Site B. * indicates period of eradication of the infected plant.

Persistence of inoculum in water (Site A)

Inoculum detection frequency in water also showed seasonal patterns with highest frequency generally occurring in winter and spring and lowest in summer (fig. 2). Monitoring of inoculum in watercourses in the U.S. shows similar trends, with reduced detection during the summer months (Tjosvold and others 2002). Although detection frequency at Site A was shown to fluctuate seasonally and persist in water over a period of three years post-eradication of the outbreak, frequency did decline over time and no new plant infections occurred during the period of monitoring. The significance of detection frequency in water and level of risk posed remains unknown, but a positive correlation between detection frequency and inoculum density is assumed.

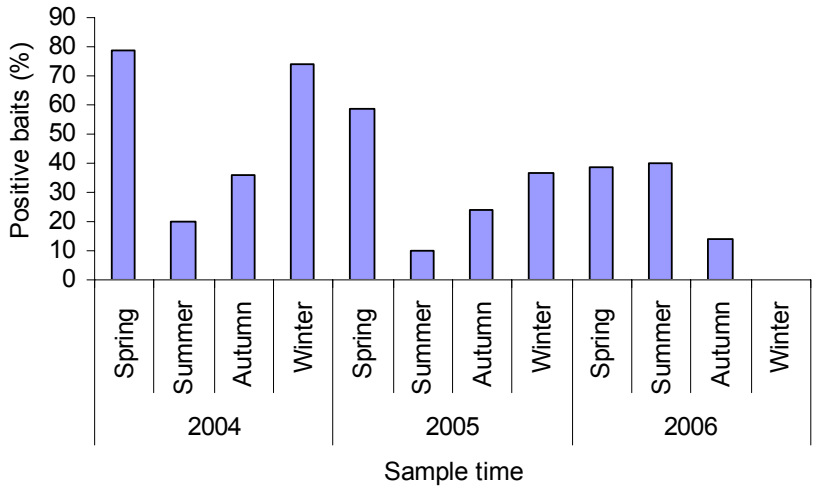

Figure 2—Detection of *P. ramorum* in water courses at Site A.

Spore Dispersal During Rainfall (Site C)

Analyses of water samples from rain traps placed at ground level at Site C indicated that splash-borne inoculum of both *P. ramorum* and *P. kernoviae* was detectable throughout the monitoring period between March and December 2006. Comparison of data on the two pathogens from all monitoring sites indicates that *P. ramorum* was more frequently detected than *P. kernoviae* as splash-borne inoculum whereas *P. kernoviae* was more frequently detected than *P. ramorum* in wind-driven rain.

Analyses of the samples from the high level traps showed that, whereas *P. ramorum* was detected in wind-driven rainfall during December only, inoculum of *P. kernoviae* was detected in May and June, absent from July to September and then detected again between October and December.

Between October 2006 and February 2007, quantitative PCR methodologies were used in conjunction with bait tests to analyse samples from high-level rain traps. The more sensitive technique showed that very low numbers of spores could be detected in rainwater samples that had tested negative using the bait tests. Quantitative monitoring at five locations within Site C showed that *P. ramorum* inoculum density peaked in December whilst density of *P. kernoviae* peaked in either November or December depending on location. It was estimated that a maximum of 40 spores of *P. ramorum* and 8000 spores of *P. kernoviae* per litre of rainwater were detected during peak months. Quantitative analysis of samples from a rain-trap located at a distance of more than 50m from an infected host also detected inoculum of both pathogens at very low levels (fig. 3). Though Brasier and Jung (2006) have observed that some infections in the U.K. could only be explained by inoculum dispersing to distances of over 50 m, this is the first report of long distance dispersal of spores of *P. ramorum* and *P. kernoviae* in the U.K. The significance of these inoculum densities in terms of disease risk in the U.K. is being investigated.

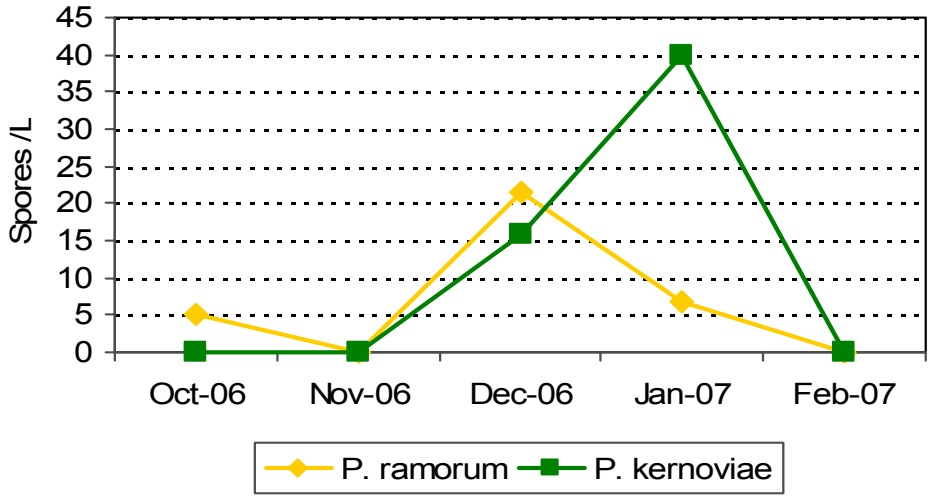

Figure 3—Detection of spore dispersal in wind driven rain in a rain trap located at a 50 m distance from infected plants at Site C.

Monitoring data from this project continue to be used to support U.K. risk analyses and development of policy. Important evidence on the benefits of quick and thorough action has assisted in advising landowners on appropriate courses of action. Data indicate that quantitative methodologies offer significant opportunities to investigate pathogen epidemiology, particularly in situations like the U.K. where the inoculum densities appear currently to be relatively low.

Acknowledgments

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Quantification of Sudden Oak Death Tree Mortality in the Big Sur Ecoregion of California[1]

Douglas A. Shoemaker,[2] Christopher B. Oneal,[2] David M. Rizzo,[3] and Ross K. Meentemeyer[2]

Abstract

Big Sur is one of the most ecologically diverse regions in California and well recognized as a biodiversity hotspot for global conservation priority. Currently the region is experiencing substantial environmental change due to the invasion of *Phytophthora ramorum*, the plant pathogen causing the forest disease known as sudden oak death. First confirmed in 2000, *P. ramorum* has spread quickly through many canyons in Big Sur killing large numbers of ecologically important oak (*Quercus* sp.) and tanoak (*Lithocarpus densiflorus*) trees with little indication of slowing. Despite these impacts detailed data on the current extent and magnitude of tree mortality are lacking yet critically needed to guide management strategies in both impacted and uninfected forests.

In this study we quantified tree mortality across a 794 km^2 area on the Pacific slope of the Santa Lucia Range in the Big Sur ecoregion. Two primary host habitat types (redwood-tanoak forest; mixed oak woodland, which includes coast live oak, shreve's oak, and black oak) were mapped in a GIS from high-resolution (0.33 m) digital color aircraft imagery collected in 2005. We also used the imagery to detect and map the location of every dead tree that exhibited spectral characteristics of trees killed by sudden oak death within each host habitat type. We evaluated the accuracy of the remote detection mapping by assessing causes of tree mortality in 77 field plots (50 X 50 m, 0.25 ha) stratified by host habitat type, oak mortality level, latitude, and fire history. Intersection of the field and remote assessments in the GIS showed that the remote mapping systematically underestimated the actual number of trees killed by sudden oak death. Tree mortality was adjusted for each host habitat type using regression models that related the field-determined number of *P. ramorum* caused deaths to the density of dead trees detected remotely and local habitat conditions. The models significantly improved remote assessment of oak mortality, with stronger relationships in mixed oak woodlands ($r^2 = 0.77$) than redwood-tanoak forests ($r^2 = 0.66$). Using these field data, we also modeled the amount of dead tree basal area (m^2) in relation to the density of mapped dead trees in mixed oak woodlands ($r^2 = 0.73$) and redwood-tanoak forests ($r^2 = 0.54$). Application of the regression models in the GIS estimated a total of 235,678 trees (12,650 m^2 tree basal area) killed by *P. ramorum* in the ecoregion, with 63 percent of mortality occurring in redwood-tanoak forests and 37 percent in mixed oak woodlands. Tree mortality of this magnitude and geographic scale has never been reported for sudden oak death. The resulting maps are being used to prioritize management strategies and examine factors driving spatial dynamics of disease spread in the region.

Key words: *Phytophthora ramorum*, forest disease, tree mortality, remote sensing, GIS, landscape epidemiology, Big Sur.

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Distribution of *Phytophthora ramorum, P. nemorosa,* and *P. pseudosyringae* in Native Coastal California Forest Communities[1]

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Abstract

Phytophthora ramorum, causal agent of sudden oak death, is well established over approximately 450 km of native forest along the California coast. In the course of research on this invasive exotic pathogen, two other putatively exotic aerial *Phytophthora* species, *P. nemorosa* and *P. pseudosyringae*, were discovered (Ivors and others 2004, Linzer and others 2006). Little is known about the ecology and biology of these other species and how they interact with *P. ramorum*. Preliminary research has found that *P. nemorosa* and *P. pseudosyringae* have similar host and geographic ranges and cause similar disease symptoms as *P. ramorum* (Hansen and others 2003, Murphy and Rizzo 2006, Wickland and Rizzo 2006). However, *P. nemorosa* and *P. pseudosyringae* do not appear to cause landscape level mortality of oaks (*Quercus* spp.) or tanoak (*Lithocarpus densiflorus*) and infect fewer plant species, as does *P. ramorum.* Additionally, while all three pathogens are patchy over the landscape, *P. nemorosa* and *P. pseudosyringae* are distributed over a broader geographical area than *P. ramorum,* extending into the Sierra Nevada. Symptoms caused by these three species are indistinguishable in the field and the causal species can only be identified using either molecular methods or microscopically once cultured.

A plot study was established to determine the distribution and incidence of *P. ramorum*, *P. nemorosa* and *P. pseudosyringae* in coastal forest communities, and to relate pathogen presence to community, structural, and environmental variables. A total of 499 circular, 1/20 ha (500 m^2) plots were established at 38 sites throughout central and northern coastal California with 2 to 38 plots per site (Fig. 1). Field plots were installed during the spring and summer months between 2001 and 2005. The majority of sites are in national, state, county, and regional parks with several sites on university reserves and private properties. Plots were established within four native forest alliances: coast redwood, coast live oak, mixed oak, and Douglas-fir-tanoak forests (Sawyer and Keeler-Wolf 1995). Each of these forest communities contains numerous hosts of *P. ramorum* and at least two hosts of *P. nemorosa* and *P. pseudosyringae*. California bay laurel (*Umbellularia californica*) is the only species that overlaps all four plant communities. All three pathogens have the potential to spread and effectively infest all four forest communities and essentially interact within the same ecological niche. It is important to note that California coastal forests are very heterogeneous with many ecotones, so transitions between forest types can occur rapidly, contributing to the patchy distribution of these pathogens. Plots were placed in a stratified random design and indiscriminately regarding presence or absence of *Phytophthora* species. The locations were selected to represent a broad geographical range, a variety of aspects and forest community types, and areas with minimal human disturbance. Each plot was evaluated for plant species

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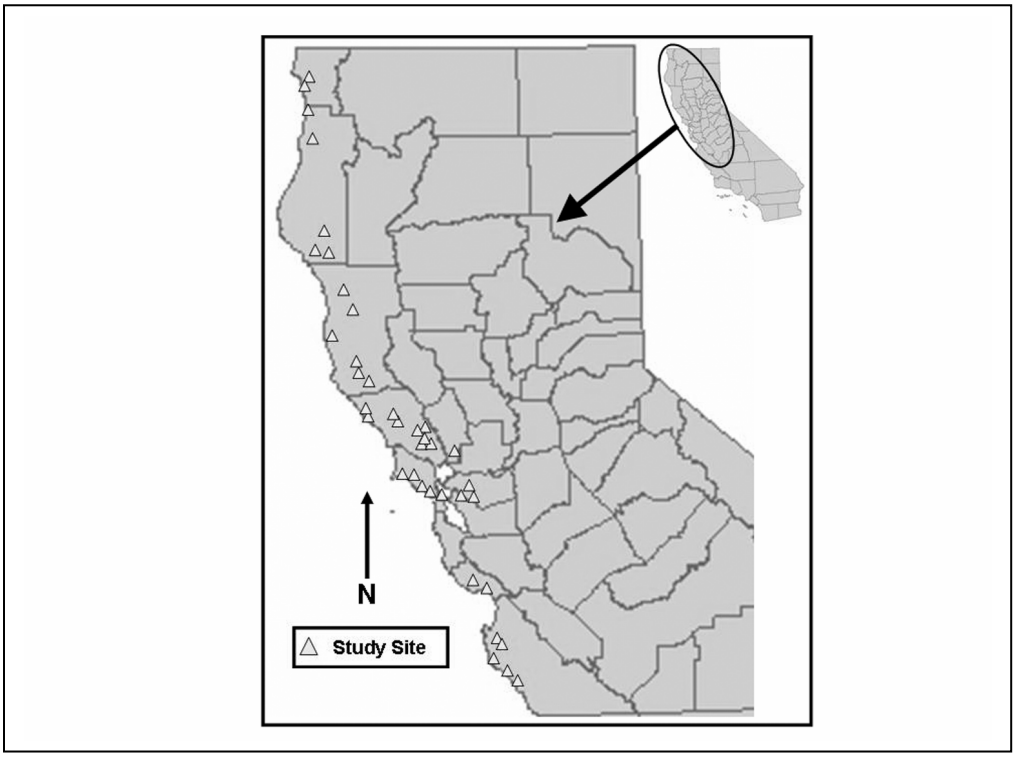

Figure 1—Map of all study sites in California where each triangle represents a single sample site. Polygons within the state represent county line designations.

composition, forest structure and environmental variables, and incidence of aerial *Phytophthora* species. Over 21,000 trees and shrubs were measured and examined for presence of aerial *Phytophthora* species. Any symptomatic aerial plant tissue (leaf or twig/trunk cankers, up to 10 to 15 leaves or canker pieces per individual) was collected and plated onto *Phytophthora*-selective medium (PARP). Plates were incubated at 20°C and checked microscopically twice weekly for one month to visually confirm presence of *Phytophthora* species.

Phytophthora ramorum was recovered from 40 percent of plots at 22 sites, with *P. nemorosa* and *P. pseudosyringae* recovered from 18 (16 sites) and 13 (11 sites) percent of plots, respectively. *P. ramorum* and *P. nemorosa* were found together on 55 plots, and were both isolated from the same tree 137 times, primarily on bay laurel (75.2 percent) but also on coast redwood (*Sequoia sempervirens*) (14.6 percent), tanoak (9.5 percent), and Douglas-fir (*Pseudotsuga menziesii*) (0.7 percent). *Phytophthora ramorum* coexisted with *P. pseudosyringae* on nine plots at five sites. These two species were isolated from the same bay laurel tree on six different plots. All three *Phytophthora* species were recovered on the same plots and from the same bay laurel tree twice at different sites. Sixteen sites were free of *P. ramorum* and include nine sites that are within 25 km of *P. ramorum* infestation. No *Phytophthora* species were detected on 220 plots and eight sites were pathogen free. Five of the sites that were *Phytophthora* free also had no bay laurel present. *P. ramorum*, *P. nemorosa,* and *P. pseudosyringae* were found in all four forest alliances. No other aerial *Phytophthora* species were identified during the course of this study.

A classification and regression trees (CART) analysis was used to identify potential pathogen predictor variables. CART analysis, a type of non-parametric statistical method, has advantages in ecological studies due to its ability to handle non-linear data well and not be constrained by distribution assumptions (Breiman and others 1984, De'Ath and Fabricius 2000). CART is a method used to explore differences among groups. The goal with CART is

to partition data into homogeneous groups, creating a tree-like classification key to predict probabilities of a response variable by selecting the most explanatory predictor variables. This objective is achieved by "growing" an overly large tree, which is then "pruned" to a parsimonious size using a cross validated cost complexity approach. CART results are visually intuitive, making them ideally suited to analyze complex ecological data.

In this study, many environmental, forest structure, and plant community variables were used to create models to predict a probabilities of each *Phytophthora* species presence or absence. The selected CART models predict the greatest probabilities of *P. ramorum* occurring at sites closer to a source of *P. ramorum* inoculum, those with greater average winter or spring precipitation, sites with any bay laurel present, and those with more moderate climates (lower maximum annual temperatures, lower solar radiation, and higher minimum annual temperatures). Similarly to *P. ramorum*, reduced annual maximum temperature, increased bay laurel abundance, and greater winter precipitation all predicted greater probability of *P. nemorosa* presence. However, reduced annual minimum temperature additionally predicted greater *P. nemorosa* presence. This concurs with lab studies that have demonstrated this pathogen's lower temperature optimum than the other *Phytophthora* species (Hansen and others 2003). In contrast to *P. ramorum* and *P. nemorosa*, the major branch for the selected CART tree predicting *P. pseudosyringae* presence was reduced average winter precipitation. *Phytophthora pseudosyringae* has been shown to be associated with drier plant communities (for example coast live oak forest type) and sites further inland from the coast (Wickland and others, unpublished).

As with previous studies, increased abundance and density of bay laurel (calculated as importance value [IV]), a reservoir host that supports high sporulation, was found to increase the probability of *P. ramorum* occurrence; a similar relationship with bay laurel was noted for *P. nemorosa* and *P. pseudosyringae* (Maloney and others 2005, Murphy and Rizzo 2006, Wickland and Rizzo 2006). Pathogen presence was highly related to forest structure, climate, and biophysical variables, although the patterns varied by species. While the probabilities of all three pathogens increase with more bay laurel present, they differ in their responses to various climatic variables, including precipitation and temperature (table 1).

Table 1—Summary of selected CART model predictor variables and the condition that increases the probability of presence of the corresponding aerial *Phytophthora* species. The selected predictor variables occur most often between the selected models for all three *Phytophthora* species. Pr= *P. ramorum;* Pn= *P. nemorosa;* Pps= *P. pseudosyringae*

Selected CART Variable	*Variable Condition*	*Pathogen*
Bay Presence (IV)	High	Pr, Pn, Pps
Winter/Spring Precipitation	High	Pr,Pn
Winter Precipitation	Low	Pps
Maximum Annual Temperature	Low	Pr,Pn
Minimum Annual Temperature	High	Pr
Minimum Annual Temperature	Low	Pn
Summer Solar Radiation	Low	Pr

While the three *Phytophthora* species occupy similar host and geographical ranges as well as the same forest communities, they differ in their specific ecological niches and impacts on coastal forests. Results from this study provide additional information about the distribution of *P. ramorum*, including location and intensity of sudden oak death within state and regional parks, as well as initial distribution information about *P. nemorosa* and *P. pseudosyringae*. This is the first study to examine the ecological associations between these three *Phytophthora* species, across a wide geographic distribution and within several forest communities. These plots are a portion of a permanent plot network that will continuously monitor pathogen infestation, and address future research questions regarding forest dynamics and ecological impacts as a result of these pathogens.

Key words: *Phytophthora ramorum*, forest *Phytophthora* species, sudden oak death, forest ecology, invasive species, California forest communities, classification and regression trees, CART analysis.

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Detecting *Phytophthora ramorum* and Other Species of *Phytophthora* in Streams in Natural Ecosystems Using Baiting and Filtration Methods[1]

Jaesoon Hwang,[2] Steven W. Oak,[3] and Steven N. Jeffers[4]

Abstract

Phytophthora spp. occur widely in forest and other natural ecosystems. Because these straminipiles are well adapted to aquatic environments, monitoring strategically selected streams may reflect occurrence and distribution of *Phytophthora* spp. over the relatively large area drained by these streams. The mountain region of western North Carolina, in the southern Appalachian Mountains, was designated as a high risk area for sudden oak death, caused by *P. ramorum*, based on the occurrence of numerous native host plants, a relatively mild climate, and the prevalence of nursery businesses in this region that import plants or plant material from areas known to be infested. Therefore, five streams in three watersheds in Pisgah National Forest in western North Carolina were sampled monthly for *Phytophthora* spp. from April 2005 to March 2006 to determine if *P. ramorum* was present in the region, to determine the diversity of species of *Phytophthora* native to the region, and to compare baiting and filtration as detection methods. For baiting, either four wounded or four non-wounded leaves of *Rhododendron maximum* (a plant native to this region) were placed in a mesh bait bag made with nylon screen and PVC pipe (fig. 1). Wounded leaves were floated in a stream for 3 days while non-wounded leaves were exposed for 2 to 3 weeks. Water soaked lesions had developed on wounded leaves after 3 days in the water, and dark brown necrotic lesions were observed on non-wounded leaves exposed for 2 to 3 weeks. In the laboratory, five pieces of symptomatic leaf tissue were taken from each leaf, a total of 40 leaf pieces were embedded in PARPH-V8 selective medium to isolate *Phytophthora* spp. for each stream. For filtration, one liter of water was collected from each stream, and samples were filtered within 10 hours of collection. Nine 100-ml subsamples of water were vacuum-filtered (fig. 2) through two types of membrane filters (47-mm in diameter) with three pore sizes (Nuclepore with 1- and 3-µm pores and Durapore with 5-µm pores), and filters were inverted on PARPH-V8 medium to recover propagules of *Phytophthora* spp. trapped on the filters (fig. 3).

P. ramorum was not found in any of the streams in western North Carolina, but *Phytophthora* spp. were detected consistently from all five streams throughout the sampling period. To date, *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. gonapodyides*, *P. heveae*, *P. pseudosyringae*, and seven morphologically and genetically distinct groups of isolates have been identified from 1560 isolates collected. *P. gonapodyides* was most prevalent (1353 isolates) and was detected consistently in all months. *P. citricola*, *P. gonapodyides*, and *P. pseudosyringae* were distributed widely and recovered from all five streams. Isolation of *Phytophthora* spp. varied depending on month, location, and detection method. Diversity of *Phytophthora* spp. was greatest in July when 11 species and groups were recovered and least in February when only one species (*P. gonapodyides*) was recovered. *Phytophthora*

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Figure 1—Nylon mesh bag used to float wounded or non-wounded rhododendron leaves in forest streams. The PVC tube provides support and buoyancy.

Figure 2—A 100 ml sub-sample of stream water was transferred by pipette and then pulled through a membrane filter with the aid of a vacuum.

Figure 3—A 47 mm diameter membrane filter on which propagules of *Phytophthora* spp. have been trapped will be inverted onto PARPH-V8 selective medium to isolate the species present.

gonapodyides and *P. pseudosyringae* were the only two species detected from November to February in all streams. The greatest diversity in a single stream occurred in the South Mills river where 10 species and groups were detected over the sample period, and the least diversity was observed in Big Creek where four species and groups were found. Over the entire study period, 13 of the 14 species and groups were detected by filtration while only eight species and groups were isolated with each baiting method. Types or pore sizes of membrane filters did not affect detection of propagules of *Phytophthora* spp. Numbers of colonies recovered from Nuclepore 1-µm, Nuclepore 3-µm, and Durapore 5-µm filters were 307, 331, and 264, respectively. Eight species and groups were trapped by Nucleopore 1-µm and Durapore 5-µm filters while nine species and groups were isolated with Nuclepore 3-µm filters.

Filtration was validated as an effective method for detecting *P. ramorum* in streams in California where this pathogen previously had been found. In May 2005, three streams in Santa Cruz county were sampled and *P. ramorum* was detected in each one. In December 2005, *P. ramorum* was detected in four of eight streams across four counties (Marin, Monterey, Santa Cruz, and Sonoma). Densities of *P. ramorum* in waterways in Santa Cruz county were significantly lower in December than in May. From Lompico Creek, 36 (51 percent) of 70 isolates of *Phytophthora* spp. detected in May were *P. ramorum* whereas only two (4 percent) of 52 isolates of *Phytophthora* spp. detected in December were *P. ramorum*. Filtration was more effective and efficient than either baiting method for detection of diverse populations of *Phytophthora* species in forest streams. Filtration also provided quantitative data on inoculum density.

Key words: Diversity, forest streams, detection, membrane filter, *Rhododendron maximum.*

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2006 Pilot Survey for *Phytophthora ramorum* in Forest Streams in the USA[1]

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Abstract

Methods for detecting *Phytophthora ramorum* and other *Phytophthora* species with rhododendron leaf baits were pilot tested in high-risk watersheds in 11 states for the purpose of recommending a national survey protocol. Ninety streams, including 14 draining *P. ramorum*-endemic areas, yielded 587 baiting chances. Molecular diagnostic assays detected the pathogen in all known *P. ramorum*-endemic streams at least once in five baiting periods, and in nearly 50 percent of all bait leaf sets. Isolation using selective media detected the pathogen in all but one known positive stream in California and Oregon, and for the first time in one Washington stream draining a confirmed positive ornamental nursery. Overall, *Phytophthora* spp. were detected by isolation in all but one stream over the course of the pilot survey, and in over 80 percent of the leaf bait sets. The national *P. ramorum* early detection survey for U.S. forests will recommend rhododendron leaf baiting for five baiting periods in high-risk watersheds with redundant molecular and selective media isolation assays for 2007.

Key words: *Phytophthora ramorum*, survey, *Phytophthora* baiting.

Introduction

Phytophthora species are well adapted to aquatic environments. The occurrence and distribution of *Phytophthora* in waterways in various environmental settings worldwide has been studied using filtration and plant tissue baits from a wide variety of plants species. Recently, monitoring of *Phytophthora ramorum* Werres, De Cock, & Man in't Veld in forest streams has been shown to be effective using pear fruits and foliage of tanoak and rhododendron in California (CA) and Oregon (OR). The success of these methods for detecting *P. ramorum* in water, even before symptoms are visible in aerial surveys, has resulted in early detection and treatment of known infested sites (Murphy and others 2006, Hansen and others 2006). Given that previously unknown infection centers have been detected up to 8 km downstream from the inoculum source, monitoring forest streams for *P. ramorum* in high-risk regions should afford an opportunity to survey larger land areas with greater efficiency and at lower cost than is possible with current terrestrial survey techniques. The objective of this pilot survey was to evaluate at a large geographic scale, existing

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stream baiting and lab diagnostic methods for the purpose of recommending protocols for the national *P. ramorum* early detection survey for U.S. forests.

Methods

Stream Selection and Bait Deployment

Pilot survey protocols were developed by consensus of researchers experienced in *Phytophthora* spp. early detection by baiting in nursery and forest environments. To achieve the desired scale of the evaluation, we targeted 11 states that contain a wide diversity of oak forest ecosystems that have been projected as high risk for *P. ramorum* establishment and damage (Oak and others 2006). Included were states with endemic areas (CA and OR); those where the pathogen had been confirmed only in woody ornamental nursery stock [Georgia (GA), Maryland (MD), North Carolina (NC), Pennsylvania (PA), Tennessee (TN), Virginia (VA), and Washington (WA)]; and those that had only received unconfirmed, but potentially infected nursery stock [Kentucky (KY) and West Virginia (WV)]. We recommended that 5 to 10 watersheds in high-risk areas of 2,000 to 4,000 ha each be surveyed, including some known *P. ramorum* positive streams in endemic areas. At a minimum, areas selected required high-risk host type and suitable climate. Where possible, a confirmed trace forward nursery inside the limits of the watershed was preferred.

Each stream was baited with four detached but otherwise unwounded *Rhododendron* spp. leaves contained in a mesh bag made of plastic window screen. Bags were tethered to the stream banks and floated in the current for one to two weeks (exposure period depended on symptom development). After retrieval, bait leaves were wrapped in a paper towel moistened with stream water, sealed in plastic bag, and placed on ice for transport to diagnostic laboratories.

Diagnostics

Bait leaves were washed under running tap water to remove silt and organic debris and blotted dry. Symptomatic leaf pieces were then excised without surface disinfestation for primary and secondary diagnostics. The primary diagnostic tested for the presence of *P. ramorum* using nested or real-time PCR according to United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) protocols (Levy and others 2004, DeVries and others 2006). The secondary diagnostic tested for the presence of any *Phytophthora* species, with the exact method left to the discretion of the diagnostician. Choices were isolation on selective medium (PARPH-V8 preferred; Jeffers and others 1987); an independent molecular diagnostic (for example Bonants and others 1997); or commercially available ELISA kits (Agdia® Elkhart, IN). After excision of bait leaf pieces, leftover leaf tissues were shipped to a separate laboratory for isolation on PARPH-V8 as validation of the *Phytophthora* spp. diagnosis in the case that diagnosticians did not choose isolation. This validation was performed only for baits from non-*P. ramorum* endemic watersheds in eastern states to prevent the inadvertent transport of the pathogen in baits.

Results and Discussion

Results for NC and TN are combined since all watersheds were within the boundary of the Great Smoky Mountains National Park, and the same field crew and laboratory handled field sampling and diagnostics. Overall, 90 watersheds were sampled for the

requisite five baiting periods outlined in the protocol, or more. These 90 watersheds afforded 587 baiting chances, of which fewer than 7 percent were lost due to high storm flows, vandalism or other causes (table 1). The highest rate of loss was in WV, where a single storm event in June disrupted all bait stations. The average number of baiting chances per stream overall was about six, with a range of four (VA) to 11 (WA).

Table 1–Pre-Survey *P. ramorum* status and bait retrieval results

	Number of Streams			
State	Total	*P. ramorum* Positive	Baiting Chances	Lost Chances[1]
CA	10	8	50	0 (0.0)[2]
OR	12	6	121	4 (3.3)
WA	11	0	129	10 (7.8)
GA	10	0	50	0 (0.0)
MD	9	0	48	6 (12.5)
NC-TN	10	0	50	7 (14)
PA	10	0	49	3 (6.1)
VA	7	0	27	0 (0.0)
KY	6	0	33	3 (9.1)
WV	5	0	30	6 (20)
Total	90	14	587	39 (6.6)

[1]Lost bait sets not available for diagnosis due to high storm flows or vandalism.
[2]Numbers in parentheses are percentage of total baiting chances for each state.

Previous sampling showed that *P. ramorum* was present in 14 of 22 watersheds in CA and OR. Nested PCR applied in CA detected *P. ramorum* in over 75 percent of the bait leaf sets from known positive streams, while real-time PCR applied in OR detected the pathogen in less than one-third (table 2). This could be due to differences in the relative sensitivity of the assays, or to lower rates of bait colonization in OR where inoculum density in streams draining *P. ramorum*-endemic areas undergoing eradication treatments may be lower than in infested streams in CA. The pathogen was detected in baits from all known positive streams at least once over the prescribed five baiting periods in both states. Real time PCR did not detect the pathogen in OR streams thought to be free of the pathogen, but nested PCR detected the pathogen in two CA streams where it was not previously known to occur. These are either instances of first detections in a previously negative stream, or false positives.

Isolation detected *P. ramorum* in all positive streams in CA at a frequency comparable to nested PCR (table 3). However, isolation failed to detect the pathogen in one known positive stream in OR, and detection rates were lower overall than for real-time PCR. The difference in detection success may reflect greater relative

Table 2—Molecular diagnosis results for *P. ramorum* in OR and CA, by pre-survey pathogen status [1]

	P. ramorum Positive Streams			*P. ramorum* Negative Streams		
State Method	No.	Diagnosis Chances	Pram+ Diagnosis	No.	Diagnosis Chances	Pram+ Diagnosis
CA Nested	8	40	31 (77.5)[2]	2	10	3 (30)
OR Real-Time	6	55	16 (29.1)	6	52	0 (0.0)
Total	14	95	47 (49.5)	9	62	3 (4.8)

[1]Streams must have had at least five bait sets with the diagnostic to be included.
[2]Numbers in parentheses are percentage of total diagnosis chances for the state/method combination.

sensitivity of the molecular assay than isolation, but could also have been due to a smaller sample size (only one-third the number of isolation chances versus real-time PCR) and low inoculum density. Isolation on selective media detected *P. ramorum* for the first time in one intermittent WA stream draining a previously confirmed positive ornamental nursery. Subsequent vegetation surveys up- and downstream did not detect any infection centers outside the nursery, and the inoculum source appears to be associated with an area where infected plants were held prior to being destroyed. The pathogen was recovered in over half of the isolation attempts overall, but these included mid- and late summer baiting periods inhospitable for the pathogen. Isolation success was over 80 percent during optimal conditions.

Isolation recovered *Phytophthora* spp. in over 80 percent of the attempts, and only one WV stream failed to yield a *Phytophthora* spp. at least once in five baiting periods (table 3). Recovery rates among states ranged from 55.8 to 100 percent

demonstrating the ubiquitous distribution of *Phytophthora* spp. in streams. ELISA assays were used only in GA and PA, and in concert with isolation in both cases. All positive ELISA results obtained in these two states were substantiated by isolation. Though unreported here in detail, molecular tools for detection of *Phytophthora* spp. in this survey were inadequate. At times, samples known positive from isolation yielded a negative molecular diagnosis. Other complications arose in PCR protocol parameters and the availability of some reagents.

2007 Early Detection Protocol

The results of this pilot survey support our recommendation of the following protocol for the 2007 national *P. ramorum* early detection survey for U.S. forests:

1. Survey up to 10 high-risk watersheds per cooperating state, as funding permits.
2. Deploy two mesh bags containing four intact, symptom-free leaves of *Rhododendron* spp.
3. Use native or naturalized source plants, if available, and ensure that they have been free of pesticides for six weeks before use.
4. Expose bait leaves in the stream current for one to two weeks, depending on symptom development.

5. Deploy bait leaves once per month for five months beginning when average daily temperature exceeds 15°C.
6. Pool baits from both bags and sort into four leaf sets to ensure that the range of symptom types is represented in each set.
7. Diagnose the presence of *P. ramorum* and any other *Phytophthora* spp. using isolation on selective medium (PARPH-V8 preferred) from one set.
8. In an independent laboratory, diagnose the presence of *P. ramorum* using PCR (real-time preferred) in the other set. Redundant *P. ramorum* diagnostics limit potential false negatives from less sensitive isolation, and false positives from very sensitive molecular assays.

Table 3—Isolation results for *P. ramorum* and any *Phytophthora* spp. for *P. ramorum* positive and all streams [1]

	***P. ramorum* Positive Streams**			**All Streams**		
State	**No.**	**Isolation Chances**	***P. ramorum* Positive**	**No.**	**Isolation Chances**	**Any *Phytophthora* spp.**
CA	8	40	33 (82.5)	10	50	40 (80.0)
OR	3	17	2 (11.8)	12	74	68 (91.9)
WA	1	13	7 (53.8)	11	109	68 (62.4)
GA	0	50	0 (0.0)	10	50	50 (100)
MD	0	42	0 (0.0)	9	42	42 (100)
NC-TN	0	43	0 (0.0)	10	43	24 (55.8)
PA	0	49	0 (0.0)	10	49	35 (79.5)
VA	0	27	0 (0.0)	7	27	26 (96.3)
KY	0	30	0 (0.0)	6	30	30 (100)
WV	0	24	0 (0.0)	5	24	17 (70.8)
Total	12	335	42 (60.0)[3]	90	498	400 (80.3)

[1]Only streams with at least five isolation attempts are included.
[2]Number in parenthesis is the percentage of isolation chances for the state/*P. ramorum* stream status combination.
[3]Percentage calculated from isolation chances in *P. ramorum* positive streams only.

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The OakMapper WebGIS: Improved Access to Sudden Oak Death Spatial Data[1]

K. Tuxen[2] and M. Kelly[2]

Abstract

Access to timely and accurate sudden oak death (SOD) location data is critical for SOD monitoring, management and research. Several websites (hereafter called the OakMapper sites) associated with sudden oak death monitoring efforts have been maintained with up-to-date SOD location information for over five years, providing information and maps of the most current spatial and attribute data on *Phytophthora ramorum* distribution in California to the public, the California oak mortality task force, and researchers. In addition to the spatial locations of *P. ramorum*, the OakMapper websites maintain a range of supporting spatial data. There are two main avenues for which to view the data: static SOD maps (state, county, and local area vicinity), and the interactive and dynamic OakMapper websites.

The OakMapper database, the statewide repository for all positive *P. ramorum* confirmations in California, has several components. The OakMapper webGIS application allows people to view confirmed *P. ramorum* trees, general SOD areas, symptomatic SOD trees submitted via an online form ("SOD sightings"), geo-located photos of SOD, United States Department of Agriculture-Forest Service (USDA-FS) annual aerial survey polygons depicting forest mortality (listed under "Aerial Survey Data"), host species areas, federal, state, and regional parks, highways, interstates, and local roads, state-wide SOD risk model created by Sonoma State University (SSU), and U.S. Geological Survey (USGS) topographical data. With OakMapper, the user can zoom in/out, query data, and search for SOD near an address. The user can link to climate data, which are long-term averages of monthly temperature and total precipitation, for each SOD confirmations, and export and print custom maps. Geographic layers can be turned on and off to customize the view. In addition, all the information behind the geographic data can be queried in order to learn more about each SOD confirmation.

OakMapper visitors can choose to view the OakMapper in three formats. First, they can use the original OakMapper, built with ESRI's ArcIMS, which contains numerous tools for map viewing and querying. Second, they can use the OakMapper Google Maps, where they can view SOD confirmations with easy-to-use Google Maps interface and data as the backdrop. Click on "Map" to view the road map backdrop, "Satellite" to view aerial and satellite photos as backdrops, and "Hybrid" to view both. Third, they can use the OakMapper-Google Earth, where they can view SOD confirmations and a few other data layers over the interface. Users need to install Google Earth onto their computers before they are able to view this data. Once they have it installed, they can click on the link on oakmapper.org and open the file up in Google Earth. Zoom around and tilt the earth to see a bird's eye view of SOD. In some places, you can even see the affected dead/dying trees behind the SOD confirmation! Visitors to the OakMapper can submit an SOD sighting, which is a symptomatic tree that is potentially infected with *P. ramorum*. The submitted points are then mapped as part of the OakMapper. In addition, we have been collecting user opinions about the OakMapper through an online

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survey, where users can leave valuable feedback about what they like or dislike about the OakMapper, and give suggestions on its improvement.

Key words: *Phytophthora ramorum*, sudden oak death, WebGIS, OakMapper.

Introduction

Access to timely and accurate information on the locations of trees confirmed to be infested with *Phytophthora ramorum* is critical for SOD monitoring, management and research. Such location information is commonly stored in Geographic Information Systems (GIS), and when linked with the Internet, these tools can provide support for a range of monitoring and management efforts. For example, the data depicting the most current distribution of the disease in California has been used to determine zones of infestation, to map potential climactic niches for the pathogen, and for education and outreach efforts.

We maintain several websites associated with sudden oak death monitoring efforts with up-to-date SOD location information for over five years, providing information and maps of the most current spatial and attribute data on *P. ramorum* distribution in California to the public, the task force, and researchers. There are two main avenues for data viewing and interaction: static SOD maps (at the state, county, and local area scale), and the interactive OakMapper website.

Materials and Methods

Development of a *P. ramorum* spatial database. Accurate disease location information remains a key component of all management efforts. When sampling trees suspected of being infested with *P. ramorum*, samplers are encouraged to use global positioning systems (GPS) to acquire the spatial location of sampled trees in the field. However, not all location data comes from GPS; we also receive disease confirmations with addresses, or photocopies of maps. Once collected in the field, samples are sent to the labs of the California Department of Food and Agriculture, University of California (UC) Davis, or UC Berkeley, and all disease presence/absence results are forwarded to our GIS lab at UC Berkeley.

The location information for positive *P. ramorum* confirmations are entered in a relational database in UTM Zone 10, NAD 83 format; if addresses or coordinates from other projections are provided, they are geo-coded or re-projected. We recommend Universal Transverse Mercator (UTM) as the initial projection because it prevents rounding errors common with the latitude/longitude (geographic) projection, and produces accurate, easily understood, and consistent area and distance measurements in the field. Data are then re-projected from UTM to the 'California Albers' projection created by the Teale Data Center GIS Lab. Additional ancillary geospatial datasets, including political and jurisdictional boundaries and forest and land cover data and both the tracks and overstory mortality polygons from annual aerial surveys of tree are also maintained in the spatial database.

Static SOD map creation and distribution. Maps are powerful and beautiful tools that can be used to easily communicate complex information. We have developed both static and dynamic tools for cartographic product creation and distribution. The sudden oak death monitoring website offers downloadable, "ready-made" static maps that depict SOD distribution for state, county, and vicinity areas, available in JPEG, PDF, and TIFF formats. These are updated as the SOD database is updated. In addition, we accept requests from users for custom maps, for example, the area around a specific address or local park. There have been over 350 map or data requests in the past five years from citizens (50 percent), academics (20 percent), and regulators (30 percent). Maps are used for a wide range of purposes, including state legislature briefings and public meetings, and have appeared in

many research publications, newspaper articles, and informational websites.

OakMapper webGIS. In addition to static paper and digital maps, we have used webGIS to create three internet-based portals to the SOD data. These are dynamic, customizable, and user-driven cartographic environments. The original webGIS application (Fig. 1), was built using Environmental Systems Research Institute, Inc. (ESRI) ArcIMS software, and remains the most feature-rich of the three sites. The original OakMapper allows user-specific interactions–including scale-dependent zooming, customized map creation, hyperlinked photography, and querying functions–with the spatial database. The original OakMapper webGIS site also allows users to report trees that might have the disease so that follow-up sampling can take place. These citizen-monitoring data are not designed to be rendered on the map as "confirmed" cases of SOD, but are guides to tell us where our education efforts are getting out, and if there are clusters of tree symptoms that might be overlooked. Ancillary data included in the system are the flight lines and mortality polygons associated with five years of U.SDA-FS survey-funded aerial surveys; host species and park boundary information from California GAP Analysis Project (University of California Santa Barbara 2007); and urban areas from the California Spatial Information Library (CaSIL 2007).

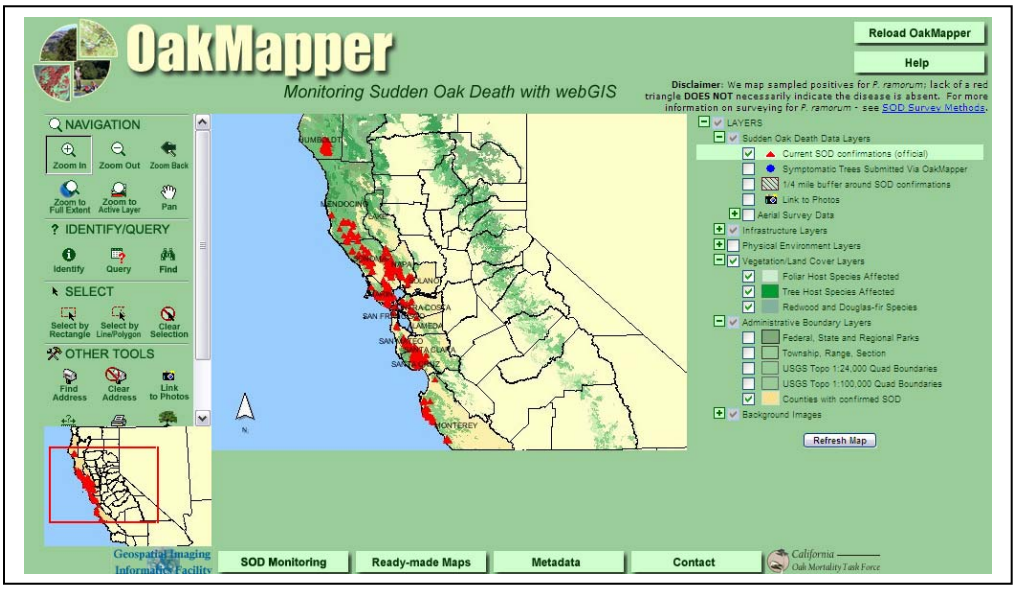

Figure 1—The original feature rich OakMapper webGIS application.

A second mode of interaction provided by the OakMapper site is powered by Google Maps ™ (Fig. 2). In recent years, several companies, including Yahoo!, MapQuest, and particularly Google via Google Earth and Google Maps have revolutionized public access to spatial data. While these on-line mapping tools are designed for a business model that focuses on advertising and marketing, the services provided are also a boon to environmental sciences. Using the Google Maps Application Programming Interface (API), we customized an OakMapper application that runs on the Google Maps server, and takes advantage of the abundance of high spatial resolution imagery in the Google Maps archive as backdrops for the *P. ramorum* confirmations. Such visualization can be very powerful at conveying the environmental niche of each confirmation, and can also be a useful outreach tool. Like the original OakMapper tool, the user need have only a web browser to interact with the data; expensive GIS software is not needed. Zoom and identify functionality are made easy through the Google Maps interface, and have proved useful for visualizing SOD confirmations over imagery with resolution high enough to view dead crowns of SOD-affected trees.

Figure 2—OakMapper webGIS powered by Google Maps.

The third model of interaction uses Google Earth ™ technology (Fig. 3). Here the user must have a copy of Google Earth (the basic version is free), or Google Earth Plus or Pro (which are not free) on their computer. Our spatial data depicting the *P. ramorum* confirmations can be added to a client-side Google Earth session, allowing seamless fly-through visualizations of the environment of each *P. ramorum* confirmation. Other data layers that exist within the Google Earth application can be viewed with the SOD confirmations; in addition, the most advanced version (Google Earth Pro) allows GIS layers on the user's computer to also be added to the layer list for enhanced GIS map-making and visualization.

Software and Hardware Information. The University of California at Berkeley has a site license for ESRI, Inc. products, and all SOD GIS data and maps are created and maintained using ESRI's GIS suite, including ArcGIS 9.2 and specialized extensions (ESRI, 2004a; ESRI, 2004b). The original OakMapper webGIS application uses ESRI's ArcIMS (version 9), an internet mapping service software package that uses languages like HTML and Java to provide users access to geospatial data residing on a server using any Internet browser. The OakMapper powered by Google Maps uses the Google Maps API to connect the OakMapper data with the Google Maps imagery and functionality. This API code is open-source and available for free from the Google Maps API website (Google 2007). The OakMapper powered by Google Earth uses Keyhole Markup Language (KML) to connect the OakMapper data with the Google Earth application.

The online submission form that allows users to enter symptomatic SOD sightings uses PHP programming languages to connect with our MySQL database. Reports submitted with a specific address are geocoded into a shapefile using ArcGIS and TIGER 2000 street data.

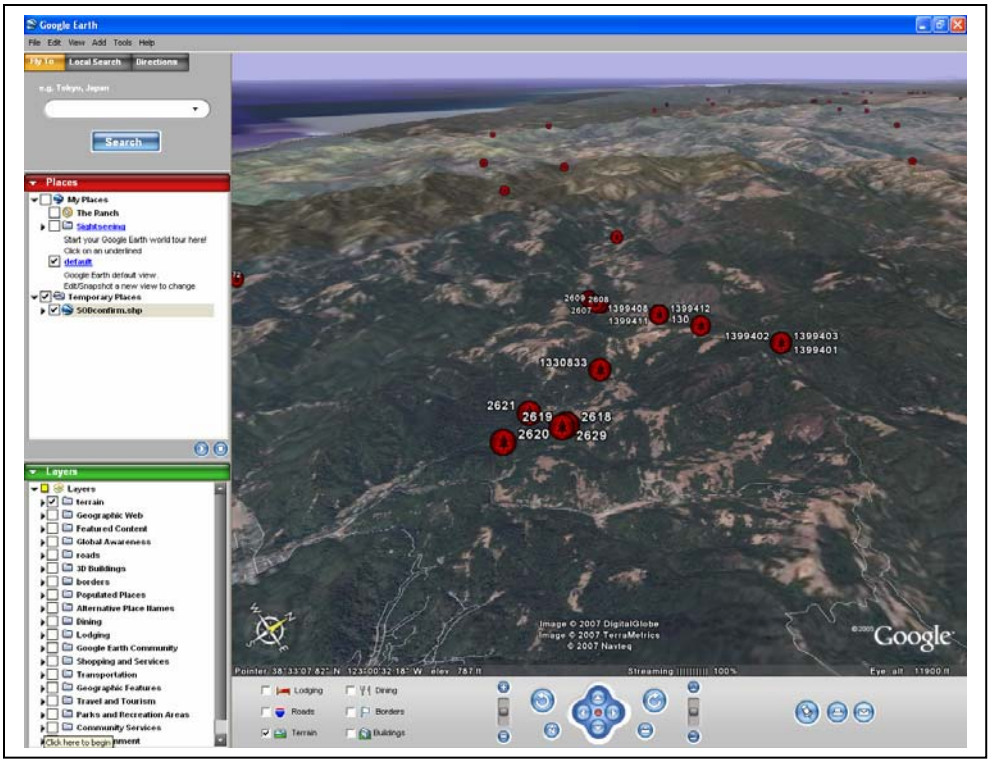

Figure 3—OakMapper webGIS powered by Google Earth.

Currently, all GIS data is stored in static vector (e.g., shapefiles, coverages) and raster (for example, GRID) formats; however, migration of all data to Arc Spatial Database Engine (ArcSDE) is planned in the near future to allow real-time updates, while also retaining the data integrity and security offered by a spatial relational database management system.

Results and Discussion

With all versions of the OakMapper, you can view the current distribution of SOD throughout California; zoom, view, and search monitoring data for both confirmed and reported SOD in California. The feature-rich original OakMapper provides additional data that the other sites do not, and allows additional queries, such as searching by street address, county, zip code, and congressional district. This site also provides additional educational material about SOD, including symptoms, host species, and monitoring updates. Also, a user can find contact information for their county's Agricultural Commissioner's office and UC Cooperative Extension office in California counties. In all versions, users can create a custom map, or snapshot of a visualization.

The data in the OakMapper database has several caveats associated with it. Privacy is a concern to many people who may have SOD on their property. With such high resolution to the backdrop imagery on the OakMapper, users can zoom in and see individual properties and houses. Oftentimes, you can see actual dead crowns on SOD-affected trees. With high-resolution data becoming ubiquitous in most web mapping application, privacy will continue to be an issue, especially surrounding environmental problems like SOD. However, as *P. ramorum* establishes itself in areas along the urban-wildland boundaries, it is less of a privacy concern affecting only a few homeowners and is more of an ecological concern affecting entire neighborhoods.

The dating of imagery is of concern to many people who use the OakMapper for decision-making. While the SOD confirmations and host layers are updated as new trees are confirmed and new species are added to the host plant list, other datasets may not be updated as frequently. Examples of these datasets include road layers, which may still consist of line files gathered from the 2000 U.S. Census TIGER data. Another example is the dates of the imagery hosted by Google Maps/Earth. While Google strives to update their image databases with the most timely and high-resolution imagery, it is important to remember that the imagery is not real-time and may have a 1 to 3 years lag time before new imagery is acquired and bought by Google to display on their applications. In addition, the dates that are displayed at the bottom of the Google Maps and Google Earth interface do not necessarily correspond with the date of imagery acquisition, and may instead indicate copyright or other image dating.

The OakMapper webGIS applications continue to be a valuable outreach tool for the sudden oak death project. We have distributed data, maps, and animated movies showing change in SOD over time to multiple people and groups. Highlights include the creation of SOD maps and animated movie for KQED's Quest online and television program (http://www.kqed.org/quest/), as well as the production of maps for a feature in the October-December 2006 issue of Bay Nature (http://www.baynature.com/v06n04/v06n04_toc.html). We have made maps for the Government Accountability Office (GAO), the USDA-F.S, Pacific Gas & Electric (PG&E), and numerous other consulting firms, government agencies, and local groups. In addition, numerous undergraduate and graduate students have requested SOD data in the past few years.

The development of web-based efforts continues to prove effective in communicating SOD information to researchers, regulators, and the general public by providing a readily available avenue for viewing, searching, querying, and exporting data and maps. The ultimate goal of the OakMapper webGIS is to empower stakeholders to participate in disease monitoring. To this end, the application is designed with non-GIS experts in mind. An online form is used to gather reports of potential SOD sightings by allowing users to: 1) select a host and visible SOD symptoms (chosen from pictures and explanations that aid in identification), 2) enter information about their familiarity with forest and forest diseases, and 3) submit the location of the tree (in other words, GPS coordinates, addresses, or location on map). The numerous submissions to date have demonstrated the success of citizen-generated data in widening the sampling effort for this disease (Kelly and Tuxen 2003). The OakMapper webGIS took more than a year to develop, and is continual work in progress as improvements are made and data is updated. We found that while a team was needed for the overall webGIS development, we needed a single champion dedicated to customizing a webGIS application for our specific project.

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Diagnostics

Lithocarpus densiflorus

Using Sigmoidal Curve-Fitting in a Real-Time PCR Detection Assay to Determine Detection Thresholds[1]

Pedro Uribe[2] and Frank N. Martin[2]

Abstract

Phytophthora ramorum, the causal agent of sudden oak death (SOD) is a quarantine pathogen that has forced the implementation of extraordinary measures to track and contain the movement of infected nursery stock both within and outside of the three western states of California, Oregon and Washington. Federal guidelines in the United States for diagnostic testing of *P. ramorum* are in place to insure the sensitivity and reliability of detection tests. PCR assays are used to determine whether the *Phytophthora* sp. detected by the initial immunoassay screening is *P. ramorum*. Most of the time definitive results can be obtained from a single PCR reaction. However, there are times when the accuracy of the results can be called into question because of low pathogen titer, a situation that can result in false negatives given the limits of detection of the marker system. In addition, experimental samples often contain significant amounts of PCR inhibitors that can also give results outside the normal detection cutoffs. Therefore the need for re-testing the samples using the same or alternative methods of pathogen detection is manifest. To understand the effect of low DNA concentration, or the role of PCR inhibitors in the extracted DNA in the sensitivity of detection of *P .ramorum*, plant and pathogen specific markers were amplified in real time PCR experiments using TaqMan® chemistry. The kinetics of amplification of the PCR reactions were modeled using a four-parametric sigmoidal curve. Standard curves of pure *P. ramorum* DNA and plant host DNA, with standard amounts of *P. ramorum* DNA added, were created and used to establish a base for data analysis. Samples having low pathogen titer were amplified and the values of the sigmoid curve parameters described. Statistical analysis of data allowed the identification of samples falling outside the proposed 95 percent confidence interval. Curve modeling also provided experimental support for determining threshold values for assessing the presence of the pathogen.

Key words: *Phytophthora ramorum*, real time PCR, PCR efficiency, 4-parametric sigmoidal regression.

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Introduction

Phytophthora ramorum, the causal agent of sudden oak death disease, is an Oomycete that can infect a large number of plant species. Given its quarantined status, special measures need to be taken to prevent the movement of the pathogen out of infested areas and in the nursery industry. Consequently, host material needs to be checked regularly for the pathogen presence, and surveys are performed at regular intervals to track the movement of *P. ramorum* pathogen in infested areas. To accurately detect the pathogen in suspected samples, a strategy based on an initial screening by immunoassay for detection of *Phytophthora* genus-specific proteins, followed, if positive, by culturing and testing by PCR is mandated. A polymerase chain reaction (PCR) assay, which specifically amplifies a portion of the ribosomal internal transcribed spacer (ITS) DNA of *P. ramorum*, is the only protocol that can be used to test samples for United States Department of Agriculture (USDA)-Animal and Plant Health Inspection Service (APHIS) regulatory purposes. Given the high importance that accurate pathogen detection has in the quarantine process, other PCR assays have been developed. Although the USDA-APHIS approved PCR tests have been optimized, the specificity and sensitivity of the assays might be variable when the quantity of the pathogen in the host sample is very low or close to detection limits. In addition, inconclusive results could be observed when mixed *Phytophthora* infections are present or when DNA contaminants that co-elute with the sample DNA during extraction affect the performance of the reactions.

In current real time PCR procedures to detect *P. ramorum* there is a cut-off value for sample amplification (Cycle threshold or Ct), above which, the sample requires a retest or is considered negative (in other words, when the signal related to specific pathogen amplification is not seen before a set number of cycles into the PCR reaction). This cut-off value is the product of the assay limits of detection and therefore a sample with very low amounts of *P. ramorum* DNA, or containing a mixture of *P. ramorum* and other *Phytophthora* species DNA or containing PCR inhibitors could be erroneously called negative. To address these possibilities in real time PCR experimentation, we used a mathematical approach based on a 4-parametric sigmoidal curve (Tichopad and others 2002) (fig. 1) to model the kinetics

$$F = Yo + \frac{a}{1 + e^{-\left(\frac{X - Xo}{k}\right)}}$$

Figure 1—4-parametric sigmoidal curve (Tichopad and others 2002). Absolute net fluorescence (a), background fluorescence (Yo), first derivative of function (Xo) and slope of the sigmoid curve (k).

of amplification of *P. ramorum*-specific real time PCR reactions (fig. 2a). Experiments were designed to test the models under ideal, in other words pure pathogen DNA or real, in other words field samples (fig. 2b), with variable amounts of pathogen DNA. Statistical analysis of the sigmoidal curve parameters of the data allowed the discovery of relationships that could yield statistical support for the identification of aberrant PCR reactions, which did not follow the specific kinetics of known *P. ramorum* amplifications. A statistical approach to determine the detection limits of the assays is proposed.

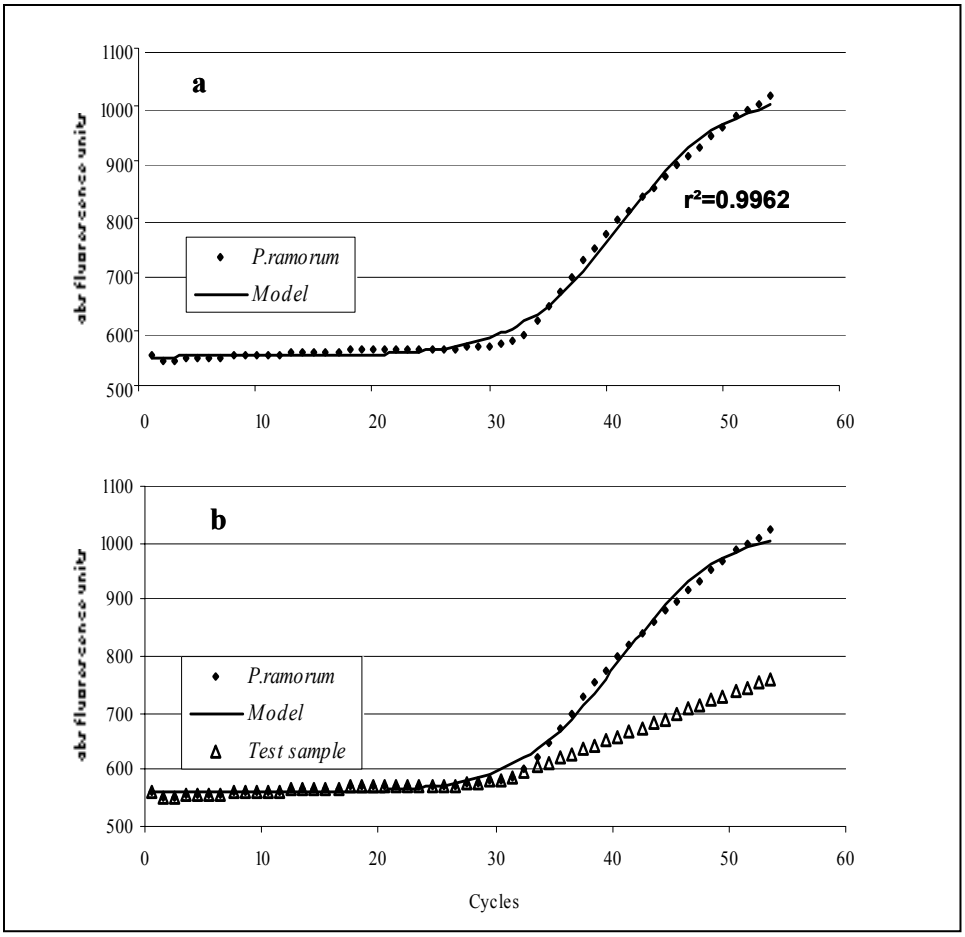

Figure 2—Standard plot of real time PCR amplifications. (a) Curve fit between sigmoidal curve model and the fluorescence output of a 20pg/µl of purified *P. ramorum* DNA. (b) *Phytophthora ramorum* suspected sample from the field (triangles) showing different PCR kinetics from the standard curve.

Materials and Methods

To extract the *P. ramorum*-infected and not infected tanoak DNA needed for the analyses, sample leaves were processed according to the official APHIS protocol for DNA extraction, based on the Dneasy DNA extraction kit from Qiagen (USDA-APHIS). Pure *P. ramorum* DNA from cultures was extracted according to Tooley and others 2004. Real time PCR amplifications were carried out in an I-cycler thermal cycler (BioRad Co., Hercules, CA) using TaqMan® master mix reagents (Applied Biosystems, Foster City, CA). *Phytophthora ramorum* specific primers

FMPr1a and FMPr7, amplifying a portion of the spacer region between CoxI and CoxII genes of the mitochondrial genome of this species (Tooley and others 2006), were used at a concentration of 1000 nM each. Plant specific primers FMPl2b and FMPl3b (Tooley and others 2006) were used at a concentration of 40 nM each as internal control.

To specifically detect pathogen amplification a TaqMan® probe, PrFAM (Tooley and others 2006), containing the fluorescent label FAM and the black hole quencher (BHQ1) was used at 400 nM. To detect plant DNA, the plant-specific probe, Plant Calred (Tooley and others), that carries the fluorescent reporter CalRed and the black hole quencher (BHQ2) was added at a concentration of 200 nM. Labeled probes were synthesized by Biosearch Co. (Novato CA) and primers by Invitrogen Co. (Carlsbad CA). All the PCR reactions included an additional 1 mM $MgCl_2$ in the standard master mix and were carried out in a final volume of 25µl. Amplification conditions used were: initial denaturation for 10 minutes at 95 °C and 55 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. A total of seven independent experiments were performed. Each experiment included an equal concentration of host DNA (tanoak) to which a dilution series of *P. ramorum* DNA had been added. Within each experiment, each sample was run in triplicate.

Pathogen concentration was initially spectrophotometrically determined using a Nanodrop (Nanodrop technologies, Wilmington DE). A total of six, 10-fold dilutions with concentrations between 200 pg/µl and 2 fg/µl were included in the experiments. To normalize PCR variability from experiment to experiment an additional standard of known concentration (50 pg/µl), called the calibrator sample, was added to the experimental set up. Threshold cycles of detection (**Ct**) were determined using the I-cycler software. For this purpose the baseline for detection was automatically set in each experiment and **Ct**s were normalized between experimental replicates by setting the software's threshold level of amplification to a value, that although different in each experiment, resulted in equal threshold cycle of detection in all the calibrator samples. Background subtracted real time PCR data was collected from the I-cycler output file and recorded in Excel (Microsoft, Redmond, WA, version 9.0 SP-3) spreadsheets and exported into Sigma Plot (Systat Software Inc, San Jose, CA, version 8.0) for modeling. Modeling process consisted in 100 iterations of the 4-parameter sigmoid curve formula (Tichopad and others 2002.). Sigmoid curve coefficients net fluorescence (**a**), slope of the sigmoid curve (**k**), first derivative of function (**Xo**), background fluorescence (**Yo**) and coefficient of determination ($\mathbf{r^2}$) were recorded and exported into Sigma Stat (Systat Software, San Jose, CA, version 3.0) and Excel software for data analysis and Sigma Plot for creating graphs. Using the values of ***Xo*** and ***k*** the initial efficiency of the reactions according to the sigmoid model (***Eo***) was calculated with the formula presented by Rutledge and others 2004 (fig. 3).

$$Eo = \frac{1 + e^{(1 + Xo / k)}}{1 + e^{(Xo / k)}} - 1$$

Figure 3—4-parametric sigmoidal curve initial efficiency formula (***Eo***) (Rutledge and others 2004).

Results and Discussion

Using background subtracted fluorescence data from replicated experiments, a database to use in the modeling of kinetics of PCR reactions was created. Each independent experiment included in the database contained the results of the real time PCR amplification of a dilution series of *P. ramorum* templates. Using Sigma Plot software, the approximation of the background subtracted fluorescence data of the reactions to the 4-parameter sigmoid curve (Tichopad and others 2002) was accomplished by repeated iterations (100 times) of the polynomial regression formula.

The values of the sigmoidal curve parameters and their corresponding coefficient of determination were recorded. The relationship between each parameter of the sigmoidal curve and the log of template concentration was compared using linear regression. Linear correlations were drawn between each of the four parameters and the log of template concentration (fig. 4). The net fluorescence values of the reaction (***a***) (data not shown) and the slope of the sigmoid curve (***k***) (fig. 4c) tended to increase with increasing template concentration, while the threshold cycle (***Ct***) (fig. 4a), and the inflection point of the sigmoid curve (first derivative of the sigmoid curve or ***Xo***) (fig. 4b) decreased with increasing template concentration. In all cases the r^2 values of the linear regression analysis were above 0.96.

Equally high r^2 values of the linear regression between sigmoid curve parameters and the log of template concentration were not obtained when the relationship between the initial sigmoid curve efficiency (***Eo***) (fig. 3) and the log of template concentration of the different standards was tested (fig. 4d). Because ***Eo*** can be expressed solely in terms of ***Xo*** and ***K***, it is expected that in experimental reactions involving identical

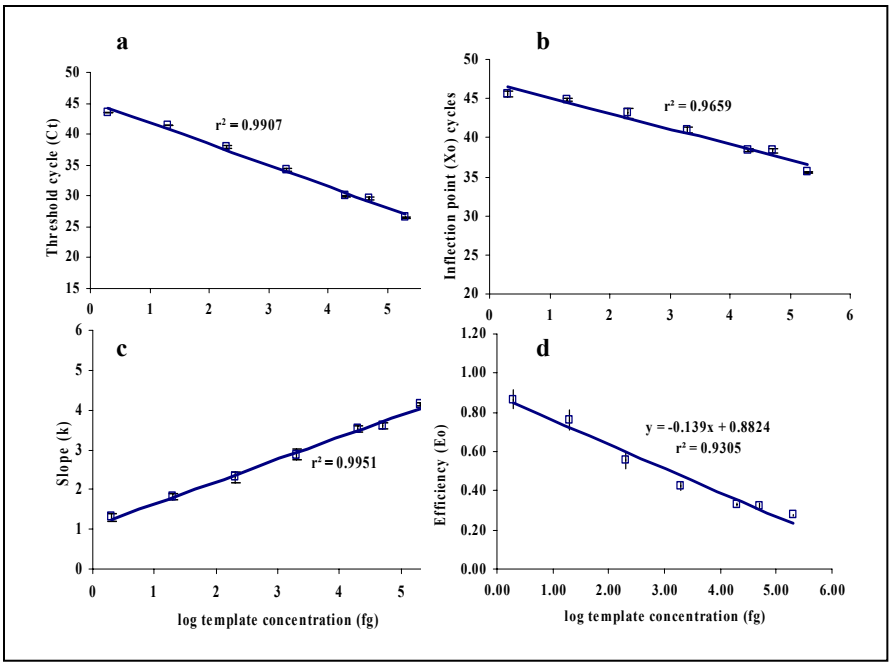

Figure 4—Linear regression analysis of four different kinetic parameters of real time PCR amplifications. (a) Threshold cycle of detection (***Ct***), (b-d) sigmoid curve parameters ***k***, ***Xo*** and ***Eo*** in repeated real time PCR reactions using standard dilutions of *P. ramorum* templates.

templates ***Xo*** and ***K*** should have very similar values and hence equal or very similar ***Eo***. (Rutledge and others 2004). Therefore by testing the behavior of ***Eo*** at different template concentrations we were able to access the effect of decreased template concentration on the value of ***Eo***. Under our experimental conditions it was possible to detect amplification of standard templates up to the fg range (2 fg/µl) with an r^2 value of 0.99 (fig. 4a) and given that in the literature it is acknowledged that ***Eo*** should be identical in PCR reactions with identical templates (Rutledge and others 2004), we expected to find similar Eo values at this detection limits of the assay.

Statistical analysis of the data using repeated measures ANOVA and Tukey tests made evident that it was possible to separate the data into two different groups. The analysis showed that at high template concentration (in other words > 3 logs of pathogen concentration or >2pg/µl) there was no statistical difference between the ***Eo*** values of the tested standards (fig. 4d). On the other hand, the data also showed that at low template concentration (in other words < 2 logs of pathogen concentration or 200 fg/µl) the ***Eo*** values of the standards were statistically different (fig. 4d). Most of the deviation between the ***Eo*** for each of the standards and their expected value (all of them equal) appeared to occur inside a range comprising the lowest template concentrations tested (data not shown), perhaps a reflection of the inability of the assay to reliably detect the presence of *P. ramorum* templates at such low concentration (detection limits).

Removing the lowest template values that were statistically different from the other higher values and comparing the relationship between ***Eo*** values and their corresponding log of template concentration with linear correlation, resulted in higher r^2 values and regression slopes closer to the expected value of 0 (Eo of equal value, fig 5). This statistical approach allowed the placing of the detection limits of the assay at the minimum standard template concentration, which would still maintain statistically equal ***Eo*** values. Using confidence interval values established for the ***Eo*** linear regression, it is possible to predict whether a field sample, after calculating its ***Eo***, will behave kinetically like the model curve.

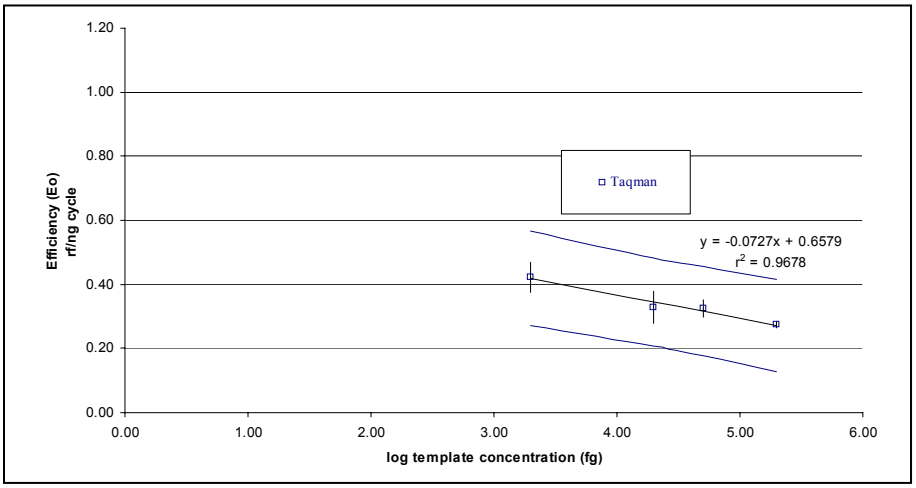

Figure 5—Amended linear regression analysis of sigmoid curve parameter ***Eo*** discarding data points shown to be statistically different in repeated real time PCR reactions from data points of greater concentrations using standard dilutions of *P. ramorum* templates. The plot also includes the 95 percent confidence intervals for analysis of suspected samples.

Using this statistically supported method it was possible to test the detection limits of the CoxI-CoxII marker system in TaqMan® real time PCR amplifications of independently determined *P. ramorum*-positive samples that were serially diluted with non-infected host tissues of equal concentration. We intentionally used this marker system instead of the USDA-APHIS approved, ITS marker system to show the usefulness and complementarity of other diagnostic systems in helping with the diagnosis of *P. ramorum*. We foresee that this methodology could be applied to any real time marker system or diagnostic technique based on real time PCR. Experiments are underway to test the system under very high template concentrations, where cross-amplification of other closely related species is observed or during mixed infections, when the interference of other DNA species can be expected. Further validation of the model is being carried out with blind samples from the field, that were independently classified as positive or negative for the presence of *P. ramorum* using the USDA-APHIS validated rDNA ITS-based PCR detection assay.

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Introducing the *Phytophthora* Database: An Integrated Resource for Detecting, Monitoring, and Managing *Phytophthora* diseases[1]

Kelly L. Ivors,[2] Frank Martin,[3] Michael Coffey,[4] Izabela Makalowska,[5] David M. Geiser,[6] and Seogchan Kang[6]

Abstract

Its virulence and ability to spread rapidly throughout the world by various means establishes *Phytophthora* as one of the most important groups of plant pathogens. Discoveries of inter-specific hybridization among *Phytophthora* species in nature, which could yield novel pathogens, further underscore the threat posed by members of this genus. The ability to accurately and rapidly identify the causal agent of a disease is crucial for developing effective regulatory and disease management strategies, and facilitates the monitoring of changes in pathogen communities as they respond to agricultural practices and environmental changes. The *Phytophthora* database (PD) (httpwww.phytophthoradb.org) project was initiated in 2005 to enhance our capability of rapid detection and diagnosis of *Phytophthora* spp. by archiving genotypic and phenotypic diversity of *Phytophthora* in a highly integrative cyber infrastructure that can easily be accessed and searched.

To establish a more comprehensive phylogenetic framework for the genus *Phytophthora*, informative molecular markers from multiple nuclear loci were identified using the available genome sequences for *P. ramorum* and *P. sojae*, in conjunction with the large number of expressed sequence tags (ESTs) available for *P. infestans*. We sequenced up to nine loci of selected isolates that represent most of the known species within *Phytophthora* (228 isolates from 80 species), (Blair and others, unpublished), including (i) two loci in the nuclear ribosomal RNA (rRNA) encoding genes: the internal transcribed spacer (ITS) regions and the 5' portion of the large subunit rRNA, (ii) nuclear genes encoding 60S ribosomal protein L10, beta-tubulin, enolase, heat shock protein 90, TigA fusion protein, and translation elongation factor 1 alpha, and (iii) a mitochondrially-encoded *coxII* gene and spacer region between *coxI* and *coxII*. The resulting phylogeny supports the division of *Phytophthora* into approximately eight major groups, and also resolves the overall relationships among these groups with moderate support. In addition to those regions, four additional mitochondrially encoded genes are being sequenced from the same isolates to construct a mitochondrially based phylogenetic framework.

To support the identification of unknown *Phytophthora* isolates via comparison of their sequences with corresponding sequences derived from archived isolates, we generated and deposited sequence data from more than 1,100 isolates representing the known diversity of

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the genus. Since ITS sequences have been commonly used for identifying oomycete species and isolates, the ITS region was sequenced from all isolates archived in the database. Although sequences for multiple loci are present for a group of isolates representing individual species, the database is currently dominated by ITS sequences. Because the PD provides applied and molecular information on *Phytophthora* species along with a suite of data analysis tools, we feel the PD has more utility than GenBank. In addition, the data stored at the PD is provided by specialists actively working with *Phytophthora*, hence proper identification of reference cultures should be less prone to error. This project is on-going; sequence and species data are continually being deposited to increase utility and breadth of this database. Scientists interested in *Phytophthora* are encouraged to use and contribute to the database.

The phylogenetic framework established at this database supports the development and validation of molecular diagnostic approaches designed primarily for species identification. The use of genotyping will greatly assist the study of newly isolated oomycetes by researchers who have limited experience in taxonomy, or by regulatory agency scientists who must quickly assess the threat of a new population and track its change and movement. The significance of this project to agricultural and forest biosecurity is the establishment of a baseline for monitoring the emergence of new and foreign *Phytophthora*. In addition, this database should serve as a model data collection system that could be easily adapted to develop databases for other important pathogen groups.

Key words: *Phytophthora* disease, invasive pathogen, genomics, population genetics, systematics, taxonomy.

Detection of mRNA by Reverse Transcription PCR as an Indicator of Viability in *Phytophthora ramorum*[1]

Antonio Chimento,[2] Santa Olga Cacciola,[2] and Matteo Garbelotto[3]

Abstract

Real-Time PCR technologies offer increasing opportunities to detect and study phytopathogenic fungi. They combine the sensitivity of conventional PCR with the generation of a specific fluorescent signal providing both real-time analysis of the reaction kinetics and quantification of specific DNA targets. Before the development of Real-Time PCR and the opportunity to provide quantitative data, the risk of false-positive PCR results due to detection of dead cells was considered only a minor setback. This, therefore, has led to a renewed interest in the risk of false-positive PCR results. In order to deal with this potential problem we developed a new reverse transcript (RT) PCR assay based on the use of mRNA as a viability marker, on the basis of its rapid degradation compared to DNA. We developed new primers, specific for *P. ramorum*, designed in the cytochrome oxidase gene encoding subunits I (COXI). To evaluate the specificity of the method, four isolates of *P. ramorum* and 11 different *Phytophthora* species were tested. One hundred symptomatic bay leaves from three different sites in California were collected in three different seasons of the year. Samples were plated on PARP selective media for *Phytophthora* and tested with the new RT-PCR method and compared with a TaqMan and SybrGreen Real-Time PCR assay after DNA extraction. Results showed that after seven days RNA of freeze-dried killed *P. ramorum* was undetectable while DNA gave a positive signal. Furthermore, data from the new assay were more correlated to the results obtained after isolation on selective media whereas DNA-based results showed more positive samples. This indicates that by using the new RT-PCR method, the risk of false-positive PCR results due to detection of dead cells can be minimized.

Key words: *Phytophthora ramorum*, RT PCR, dead cells, false positives, diagnosis.

Introduction

Phytophthora ramorum (Werres, de Cock and Man in 't Veld), causal agent of sudden oak death (SOD), is one of the best-known examples of an exotic pathogen which has killed thousands of oak trees along the U.S. west coast, causing a dramatic decline of local forests in terms of structure and biodiversity.

The development of accurate and rapid diagnostic methods for *P. ramorum* is crucial for providing the tools useful for detecting the pathogen and determining its potential for establishing itself in new regions.

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Real-Time PCR technologies offer increasing opportunities to detect and study phytopathogenic fungi. They combine the sensitivity of conventional PCR with the generation of a specific fluorescent signal providing both real-time analysis of the reaction kinetics and quantification of specific DNA targets (Schmittgen 2001). One disadvantage of DNA-based methods is that they do not distinguish between living and dead organisms, which limits their use for monitoring purposes. In order to deal with risks of false-positive PCR results, many researchers have investigated the use of mRNA as a viability marker, on the basis of its rapid degradation compared to DNA (Alifano and others 1994).

The principal objective of this research programme is to define scientific parameters to discriminate dead cells from living cells of *P. ramorum* present in plant material and develop a reliable and sensitive molecular method to assess the risk of false positive in the detection of *P. ramorum* from environmental samples.

Real-Time PCR was used to detect the expression of the cytochrome oxidase 1 gene in order to examine the relationship between viability and presence of mRNA, since any relationship between mRNA and viability may depend on the method used to inactivate cells or the type of mRNA sought. We exposed the cells to three different stress treatments (heat, lyophilization and ethanol) and assayed mRNA from the cytochrome oxidase 1 gene. Furthermore, the method was validated testing symptomatic bay leaves collected in different seasons from three sites in California. Results obtained with RT-PCR were compared with those obtained by traditional isolation on PARP selective medium and Nested TaqMan and SYBR Green PCR.

Materials and Methods

Sampling and Isolation

The survey was carried out in California and the chosen sites were: 1) China Camp State Park, which lies along San Pablo Bay near San Rafael; 2) Briones Regional Park on the eastern side of San Francisco Bay and 3) Samuel P. Taylor State Park located in Marin County north of San Francisco. Sites were selected to encompass a range of habitat types and species compositions found within *P. ramorum* infested forests.

Between October 2005 and July 2006, each site was inspected for the presence of SOD. China Camp State Park was sampled three times (October, April, July), whereas Samuel P. Taylor and Briones Parks were sampled only in July. At each inspection symptomatic leaves from 20 California bay laurel trees were randomly chosen, collected and taken to the laboratory (total of 100 leaves). Leaves were superficially wiped with 70 percent EtOH and then divided in three homogeneous sectors containing approximately the same amount of lesions and healthy tissues. Each piece, randomly chosen, was assigned to one the different treatments (direct plating on PARP selective medium, DNA or RNA extraction) in order to compare different diagnostic methods.

Asymptomatic California bay laurel leaves, collected from the University of California (UC) Berkeley campus, were used as negative control.

Inactivation Treatments for RT-PCR

In order to validate the new diagnostic approach mRNA was recovered from pure cultures of *P. ramorum* (isolates Pr1, Pr52, Pr72 and Pr102) that had been subjected to treatments designed to impact cell viability.

Isolates Pr1, Pr52, Pr72 and Pr 102 were cultured on pea broth for 7 to 10 days and than the mycelium was harvested, washed with sterile water and then treated with 70 percent EtOH for 1 hour, heat-treated at 60°C for 1 hour or freeze-dried (lyophilized) overnight in order to inactivate the cultures. Treated isolates were plated on V8A in order to monitor the presence of viable *P. ramorum* cells. All the treated mycelia were left at room temperature for 0, 1, 2, 5, 7, 9 and 12 days and then kept at -80°C before proceeding with RNA and DNA extraction.

Nucleic Acids Extraction

Mycelium and plant material was lyophilized before extractions. DNA from approximately 50 mg of mycelium was extracted using a PureGene DNA Extraction Kit (Gentra) according to the manufacturer's instructions. DNA from approximately 50 mg of lyophilized bay leave tissue was extracted according Hayden and others(2004). RNA was extracted from approximately 50 mg of mycelium or approximately 50 mg of symptomatic bay leaves using RNeasy® Plant Mini Kit (Qiagen) following the manufacturer's protocol. RNA was purified from DNA contamination using RNase-Free DNase Set (Qiagen).

Primer Sequence Design and Specificity

The COXI sequence of *P. ramorum* was utilised to design specific primers to amplify DNA fragments from this particular species. Sequences of *P. ramorum* and related species were aligned using the ClustalW software (EMBL, European Bioinformatics Institute) and screened for base pair differences and then best primer sets were designed using Primer3 software. Specificity of ACPramF and ACPramR primer set was preliminarily assessed by means of Basic Local Alignment Search Tool (BLAST) analyses to explore all of the available sequence DNA databases and exclude the presence of similar sequences in other microrganisms. Furthermore, the specificity of these primers was assessed using genomic DNA from other species of *Phytophthora* (table 1).

Two-Step RT-PCR

The presence of target COXI RNA was analyzed by two step RT-PCR using QuantiTect® Reverse Transcription Kit (Qiagen) following the manufacturer's procedure. Each purified RNA sample was briefly incubated in gDNA Wipeout Buffer (Qiagen) at 42°C for two minutes to efficiently remove any possible contaminating genomic DNA.

cDNA was then amplified using ACPramF/ACPramR primer set. PCR was performed in a total volume of 25µl containing 6.26µl of undiluted cDNA combined with 12.5µl of 2x iQTM SYBR® Green Supermix (Bio-Rad) containing 0.5 mM of each primer. Real-Time amplification was carried out in an iCycler IQ Real-Time

thermalcycler (Bio-Rad) using the following conditions: 1 cycle at 50°C for 10 minutes, 1 cycle at 95°C for 3 minutes, 40 cycles at 95°C for 15 seconds, and 58°C for 1 minute. Ramp rate was 3.3°C/s heating and 2.0°C/s cooling.

For each PCR run with SYBR Green detection, a melting curve analysis was performed to guarantee the specificity in each reaction tube (absence of primer dimers and other non-specific products).

Table 1—*Phytophthora* species used to determine specificity of the *Phytophthora ramorum* specific primers

Species	Local isolate no.	Host	Origin	Amplification
P. cambivora *	MP14	*Quercus agrifolia*	California	Not cross-amplified
*P. cambivora**	MP22	Almond	California	Not cross-amplified
*P. cambivora**	NY217	Apple	New York	Not cross-amplified
*P. cambivora**	NY249	Apple	Oregon	Not cross-amplified
*P. citricola**	MP18		California	Not cross-amplified
*P. cryptogea**	MP11	*Lycopersicon esculentum*		Not cross-amplified
*P. drechsleri**				Not cross-amplified
P. hibernalis				Not cross-amplified
*P. nemorosa**	MP16		California	Amplified >1 pg
*P. lateralis***	PL27	*Chamaecyparis lawsoniana*	California	Not cross-amplified
*P. megasperma**	MP20	*Glycine max*	Wisconsin	Amplified >1 pg
*P. palmivora**	MP8	*Theobroma cacao*		Amplified >1 pg
*P. pseudosyringae**	P40	*Quercus agrifolia*	California	Amplified >1 pg
*P. syringae**	MP15	*Rhododendron* spp.	California	Not cross-amplified
*P. ramorum***	Pr1	*Quercus agrifolia*	California	Amplified
*P. ramorum***	Pr52	*Rhododendron* sp.	California	Amplified
*P. ramorum***	Pr72	*Rhododendron* sp.	California	Amplified
*P. ramorum**	Pr102	*Quercus agrifolia*	California	Amplified

* isolates used in Hayden and others (2004). ** Isolates used in Hayden and others (2006).

Nested SYBR Green Real-Time PCR

The first and second round of PCR amplification were performed using primer sets Phyto1/Phyto4 and Phyto2/Phyto3, respectively following the procedure described by Hayden and others (2004).

Nested TaqMan Real-Time PCR

TaqMan reaction was performed using primer set Phyto1/Phyto4 (1st round) and primers Pram5/Pram6 and probe Pram7 (2nd round) following the same conditions previously described by Hayden and others (2006).

Results and Discussion

Detection of mRNA From Killed Cells

Of the three different inactivation treatments chosen, only lyophilized, *P. ramorum* did not grow when plated after the treatment. Heat-treated mycelium of *P. ramorum* remained active, whereas all EtOH-treated samples were inactivated after three weeks incubation with the exception of isolate Pr52 (two of four plates) that was still active after three months (showing sparse mycelium).

mRNA from the COXI gene was detected by RT-PCR in all the samples immediately after treatments. However, during subsequent incubation at room temperature, mRNA of lyophilize-treated samples became undetectable after seven days, whereas all other samples gave a positive RT-PCR amplification (table 2). Samples used as negative control never showed a positive amplification or any growth on plates.

Table 2—mRNA detected by RT-PCR after incubation of treated *P. ramorum* mycelium at room temperature

Treatment	Target	Incubation at room temperature *						
		0	1 day	2 days	5 days	7 days	9 days	12 days
Untreated	COXI	Y	Y	Y	Y	Y	Y	Y
60°C for 1 hour	COXI	Y	Y	Y	Y	Y	Y	Y
EtOH 70 percent for 1 hour	COXI	Y	Y	Y	Y	Y	Y	Y
Lyophilized	COXI	Y	Y	Y	Y	N	N	N

* Y, positive RT-PCR amplification; N, negative RT-PCR amplification.

Comparison of Traditional and Molecular Diagnostic Methods

One hundred bay leaves samples, showing SOD symptoms, were assayed for the presence of *P. ramorum* with four different diagnostic methods: isolation on PARP selective medium, COXI RT PCR, Nested TaqMan PCR, and SYBR Green PCR. The July survey samples that were collected in all three sites had the most xeric conditions and therefore the detection of *P. ramorum* was low with all four assays. Isolation with selective PARP medium gave three positive (15 percent) out of 20 samples, whereas molecular RNA and DNA assays gave 25 percent and 30 percent positives, respectively. All the samples collected in China Camp State Park were positive when tested with Nested TaqMan and SYBR Green PCR (100 percent), whereas RT-PCR and PARP isolation gave 45 percent and 35 percent of positives, respectively. Samples collected in Samuel P. Taylor State Park, which is considered to be the most mesic site, were 5 percent positive when plated on PARP, 50 percent when assayed with RT-PCR and 75 percent when assayed with TaqMan or SYBR Green assays (fig.1).

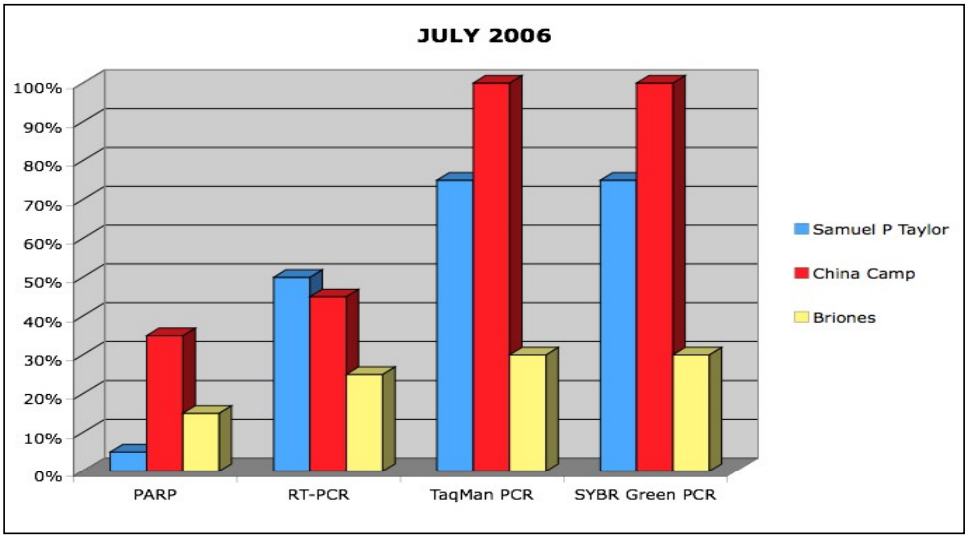

Figure 1—Comparison of four different methods to detect *P. ramorum* from symptomatic California bay leaves. Samples were collected in July 2006 from three different sites: Samuel P Taylor State Park, China Camp State Park and Briones Regional Park.

Frequency of detection of *P. ramorum* from naturally infected California bay leaves differed significantly across sites and between methods of detection. Nevertheless, both Nested DNA assays, detection via SYBR Green and DNA detection via a TaqMan probe, were equally sensitive with 78 percent and 77 percent of positive samples respectively (fig. 2).

Traditional isolation with PARP selective medium was a less sensitive and reliable assay for the detection of *P. ramorum* compared to the molecular methods. In fact, only 38 percent out of all tested samples resulted positive using this approach, which required more time and effort for the isolation and the identification.

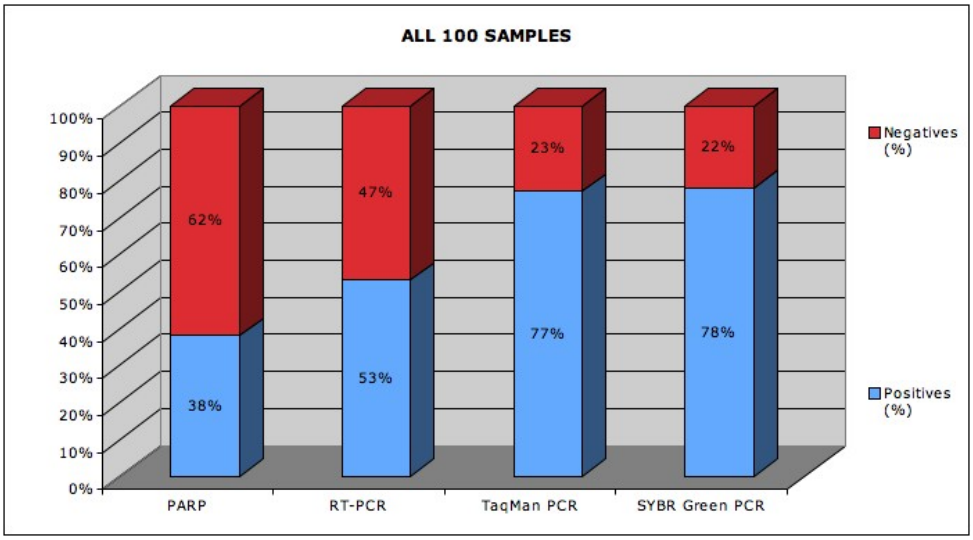

Figure 2—Comparison of four different methods to detect *P. ramorum* from symptomatic California bay leaves. Samples were collected from 100 different trees in China Camp State Park in October 2005, April 2005 and July 2006; and in Briones Regional Park and in Samuel P. Taylor State Park in July 2006.

Detection of *P. ramorum* by RT-PCR was significantly more sensitive compared to traditional isolation on PARP selective medium, but differed from DNA based detection assays with 53 percent positive out of 100 samples tested. Furthermore, all samples that were positive on PARP were positive with all three molecular methods and all RT-PCR positive samples were positive with DNA based diagnostic methods.

It should be pointed out that *P. ramorum* remained active after 1 hour at 60°C and after one hour in 70 percent EtOH. The only treatment able to devitalize *P. ramorum* was lyophilization. Results showed a good correlation between the presence of mRNA and the viability of *P. ramorum* when RNA was extracted from freeze-dried mycelium. The detection of mRNA therefore indicates either that a cell is alive or has died fairly recently. We have established that mRNA can persist for at least seven days in lyophilized mycelium of *P. ramorum*, but further analysis is required to characterize the decay rates of mRNA in dead cells in a range of conditions before the limitations of the method are fully defined. This study has demonstrated that mRNA is a promising candidate as an indicator of viability *P. ramorum*, although further evaluation with additional *Phytophthora* spp. is needed to confirm species-specificity of the diagnostic primers.

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Nursery Research and Management

Adiantum jordanii

Effect of Environmental and Seasonal Factors on the Susceptibility of Different *Rhododendron* Species and Hybrids to *Phytophthora ramorum*[1]

Isabelle De Dobbelaere,[2] Kurt Heungens,[2] and Martine Maes[2]

Abstract

Although *Rhododendron* is the most important host of *Phytophthora ramorum* in Europe, there is little scientific information about the susceptibility levels of different *Rhododendron* species and cultivars. Increasing this knowledge would help nurseries in the management of the disease and could be used by plant protection services to target their inspections. In this study a total of 80 *Rhododendron* species and hybrids were screened for their susceptibility to *P. ramorum* using two detached leaf inoculation assays. Due to the variability in susceptibility for a given cultivar within and between years, multi-year data was deemed necessary to establish a reliable susceptibility ranking. The zoospore inoculation method involving non-wounded leaves was most informative. Using this method, a wide range in susceptibility to *P. ramorum* was demonstrated. A second objective of this study was to get a better handle on some of the internal and external factors (time of year, temperature, leaf age) that seem to effect the susceptibility level. Susceptibility was significantly lower during late fall and winter, and seems correlated with the physiological status of the plant. Leaf age mainly seems to affect susceptibility during the early stages of leaf maturity. In general, new leaves were more susceptible to pathogen development. However, young leaves of some cultivars seem covered by leaf hairs, which prevent the zoospores to reach the leaf surface. Environmental factors that affect stomatal regulation, such as temperature, also seemed to have an effect on the degree of symptom development.

Key words*: Phytophthora ramorum, Rhododendron*, susceptibility, host resistance, cultivars.

Introduction

Since November 2002, EU emergency phytosanitary measures are being taken to prevent the introduction and spread of *P. ramorum* in Europe, including surveys at all commercial premises with *P. ramorum* hosts. An eradication and quarantine program is initiated at nurseries with positive findings. Commercial *Rhododendron* plants are the most important hosts of *Phytophthora ramorum* in Europe. In Belgium, one of the largest *Rhododendron*-producing countries of Europe, about 80 percent of the samples in the *P. ramorum* surveys of the plant protection service were *Rhododendron* plants. Because of the quarantine status of *P. ramorum,* only preventive measures can be taken by the growers. These measures mainly consist of

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water management, sanitation, hygiene and preventive fungicide treatments. Taking into account cultivar susceptibility would be another tool for which there is high interest, but limited availability of scientific knowledge. This information could lead to using more resistant cultivars and to extra protection of susceptible cultivars. In the long term, including resistance to *P. ramorum* may become a part of *Rhododendron* breeding efforts.

Materials and Methods

This study was initiated during the summer and early fall of 2004 with a screening of 63 *Rhododendron* species and hybrids. *Rhododendron* species were selected to represent the main subdivisions within the genus *Rhododendron*. Hybrids were selected based on their economic importance. Preliminary assays revealed no differences in pathogenicity between different *P. ramorum* isolates, hence a single Belgian isolate was used in all assays. Initially, four inoculations methods were used, involving either wounded or non-wounded detached leaves or stems. Based on the variability in susceptibility of the control cultivar over the testing period, it was concluded that multi-year data was needed to establish a reliable susceptibility ranking. Therefore, the assays were repeated in 2005 and 2006. The tests were extended to 80 *Rhododendron* hosts (24 species and 56 hybrids) but only the two most informative inoculation assays were used. A method involving non-wounded leaves and zoospore inoculation was used to estimate the ability of the host to resist tissue penetration. A method involving wounded leaves was used to evaluate the resistance to pathogen growth inside leaf tissue. Each year the *Rhododendron* plants were screened from July to September. Leaves were mature but less than one year old. The assays were performed in batches and included the same three control cultivars in each batch (*Rhododendron* Mme Masson, *R*. Cunningham's White, and *R.* Gartendirector Rieger). Seasonal effects were studied in an experiment in which leaves of *R.* Cunningham's White were collected and inoculated on a bi-weekly basis over a period of two years. During the spring, both new leaves and mature leaves of several cultivars were inoculated and compared in respect to susceptibility to *P. ramorum*. In tests involving the effect of temperature, potted plants were placed in growth chambers at 5, 17 or 28°C during several hours, after which they were used in the inoculation assay involving non-wounded leaves.

Results and Conclusions

All the *Rhododendron* species and cultivars tested were susceptible to *P. ramorum* in the method involving wounded leaves. In contrast, the method with non-wounded leaves revealed a considerable difference in level of resistance between cultivars or species. Using this method, a few *Rhododendron* species and cultivars consistently showed very low levels of disease expression. These included *R.* Gartendirector Rieger, *R.* Red Jack, and *R.* Fantastica. In contrast, some cultivars such as *R.* Mme Masson, *R.* John Walter, and *R.* Cheer were highly susceptible and may be candidates for improved *P. ramorum* leaf baiting methods. The average level of susceptibility during the screening assays was significantly different between the years but was not caused by a decrease in virulence of the test isolate. No correlation was observed between the susceptibility level and the genetic background of the hybrids. However, the genetic background of the *Rhododendron* hybrids is often mixed or unclear.

Within the *Rhododendron* species, lepidote species on average were less susceptible than elepidote species.

In terms of seasonal effects, susceptibility was significantly lower during late fall and winter, and seems correlated with the physiological status of the plant. Leaf age plays an important role in susceptibility tests. When using wounded leaves, young leaves of all cultivars tested showed a higher level of susceptibility then mature leaves. However, when using non-wounded leaves, young leaves of some cultivars were less susceptible than older leaves. This effect was correlated with the presence of hairs on the young leaves of those cultivars, which probably form a barrier to the zoospores and prevent tissue penetration by the pathogen. Exposing plants to high temperatures during several hours before collection of the leaves had a negative effect on susceptibility in most experiments. One hypothesis is that this increase in temperature results in stomatal closure, which may reduce the penetration capacity of the pathogen.

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Can *Phytophthora ramorum* be Spread With Contaminated Irrigation Water?[1]

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Abstract

In a two year study, the spread of *Phytophthora ramorum* with contaminated irrigation water and the survival of the pathogen in water reservoirs were studied (Werres and others 2007). In addition at the end of each experimental period root ball samples from asymptomatic plants were taken to look for contamination with *P. ramorum*. For the study, an open air simulation system with nine separate container stands was used. The surplus water from each container stand ran back to a separate water basin. From the water basins, the water was taken for overhead irrigation of the plants on the container stands. In both years rooted *Rhododendron* cuttings of the cv 'Cunningham's White', in the second year also *Viburnum plicatum* cv 'Mariesii' were placed on the container areas and irrigated from above with water taken from the water reservoirs. The reservoirs were inoculated once a year in June with two different inoculum concentrations (low inoculum density: 12.5 Petri dishes per reservoir = 1000 L, high inoculum density: 25 Petri dishes per reservoir in 2004 and 50 in 2005). Three reservoirs were not inoculated.

Phytophthora ramorum was able to survive in the water reservoirs during all seasons but there were differences between the inoculum densities and within the season. The pathogen could be spread with contaminated water. First disease symptoms on *Rhododendron* occurred 7 and 16 days after the first irrigation with contaminated water. The maximum incidence of *Rhododendron* plants infected visibly with *P. ramorum* was below 19 percent. The incidence varied between the two years of the study and within the season. There was a high variability in the total amount of symptomatic *Rhododendron* infected with *P. ramorum* between the three container stands belonging to the same inoculum density. The percentage of symptomatic *Viburnum* infected with *P. ramorum* was below 1 percent.

At the end of the two experimental periods when the plants were removed from the container stands, *P. ramorum* could be detected in some of the pooled root ball samples of asymptomatic plants with the bait test with *Rhododendron* leaves. *P. ramorum* was detected in the bait leaves by direct isolation and by PCR.

Key words: *Phytophthora ramorum*, sudden oak death, ramorum blight, water, nursery, survival, spread.

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Seasonal Symptom Expression, Laboratory Detection Success, and Sporulation Potential of *Phytophthora ramorum* on *Rhododendron* and *Camellia*[1]

Steve A. Tjosvold,[2] David L. Chambers,[2] and Cheryl L. Blomquist[3]

Abstract

Camellias and rhododendrons are important nursery and landscape plants and are known to be highly susceptible hosts of the quarantined plant pathogen, *Phytophthora ramorum* Werres, de Cock & Man In't Veld. Nursery inspection can not always occur during optimal conditions for the disease and its detection. The goals of this research were to (1) characterize and document seasonal development of symptoms, including, lesion growth rates and host leaf abscission, (2) evaluate the USDA-APHIS approved laboratory detection methods during four seasons, and (3) access the seasonal potential for sporangial or chlamydospore production on lesions. This paper contains the results of the first complete year of a two year field and laboratory study.

Leaves on potted *Camellia japonica* L. 'Kumasaka' plants and *Rhododendron* 'Cunningham's White' were inoculated with V8 agar plugs from *P. ramorum* cultures on 18 July 2005, 26 October 2005, 1 February 2006, and 1 May 2006, representing summer, fall, winter, and spring infections respectively. The resulting lesion growth and leaf abscission was quantified for up to 16 weeks after inoculation. Infected leaves were sampled for up to 24 weeks after inoculation. A portion of the sampled leaves were mailed by overnight courier to the California Department of Food and Agriculture Plant Pest Diagnostics Branch Laboratory where the most current federally approved methodology was used for detection (PARP semi-selective media, ELISA, and real-time and nested PCR), and detection success was evaluated. Isolations from other leaves in the sample were plated onto PARP media in a local laboratory, without shipment, and detection success was evaluated. From another portion of the sampled leaves, leaf-disks were punched from the margin of the lesions and flooded with de-ionized water. Sporangia and chlamydospores produced on the margin of the leaf-disks were identified by propagule type and counted.

Growth rates of lesions were fastest in the fall and winter and slowest in the spring. Camellia leaves abscised in significantly fewer days after inoculation in the fall and winter than in the spring and summer. Rhododendron leaves abscised infrequently after inoculation and then only in the winter. Successful laboratory detection with PARP by the State laboratory was possible with summer inoculated leaves up to 20 weeks, with fall at least up to 24 weeks, with

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[1] This paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California. It presents data but limited analysis for the first year of a 2-year study. It will become part of the completed study at the end of 2007 when data are complete and more robust analysis will be possible.

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winter up to 20 weeks, but spring only up to 4 weeks after inoculation. Laboratory detection locally (without shipment) with PARP generally mirrored this result. ELISA and PCR (nested and real time) methods were 100 percent effective in detection regardless of season or leaf age. Flooded camellia leaf-disks were capable of producing chlamydospores and sporangia 8 to 12 weeks after initial inoculation with the fall and winter more conducive to the production of these propagules than the other seasons. Flooded rhododendron leaf-disks produced sporangia for 12 to 20 weeks after inoculation and chlamydospores 4 to 20 weeks after inoculation with the fall and winter generally more conducive to the production of these propagules.

Key words: *Phytophthora ramorum*, sudden oak death, symptoms, detection, sporulation, rhododendron, camellia, nursery.

Introduction

In California, Oregon, and Washington, federal regulations require annual nursery stock inspection, leaf sampling, and laboratory analysis of host plants susceptible to *Phytophthora ramorum* for plants shipped out of state. For these regulations to be effective, agricultural inspectors must recognize disease symptoms and collect proper leaf samples, and laboratory analysis must be accurate and sensitive. Nursery inspection can not always occur during optimal conditions for the disease and its detection. If *P. ramorum* is found in a nursery and the confirmed nursery protocol is imposed, follow-up inspections and samplings could occur at nearly any time of the year. With this in mind, the goals of this research were to (1) characterize and document seasonal development of symptoms, including, lesion growth rates and host leaf abscission, (2) evaluate the United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) approved laboratory detection methods during four seasons, and (3) access the seasonal potential for sporangial or chlamydospore production on lesions. This paper contains the results of the first complete year of a two year field and laboratory study.

Materials and Methods

Ten leaves on each of 42, 3.8 L (potted) *Camellia japonica* L. 'Kumasaka' plants and 42, 3.8 L *Rhododendron* 'Cunningham's White' were inoculated with V8 agar plugs from 21 day old cultures from *P. ramorum* cultures on 18 July 2005, 26 October 2005, 1 February 2006, and 1 May 2006, representing summer, fall, winter, and spring infections respectively. The *P. ramorum* isolate used for inoculation was isolated from an infected rhododendron at a local nursery. Plants were grown under environmental conditions and with cultural practices typical of many local commercial outdoor nurseries. Plant spacing was consistent with commercial nursery practices. Plants were irrigated with overhead micro-sprinklers (Global #VS360 T), and fertilized (21-5-6 Apex®, J.R. Simplot) at a rate of 6 grams per pot every four months. All plants were placed under shade cloth (40 percent light transmission) and maintained without the use of fungicides. For each of the two species, seven plants were placed on each of six (95 cm x 82 cm x 16 cm) wooden pallets to elevate the pots well above the soil. When leaf samples were taken, they were removed randomly from each group and treated as statistical replicates.

For each seasonal inoculation and host species, the resulting uniform lesions from 30 designated leaves were digitally photographed 1, 2, 4, 8, 12, and 16 weeks after

inoculation or until the leaves abscised. The area of the lesions on each leaf was calculated with the aid of Access software (American Phytopathological Society). When available, up to 24 leaves per species were removed for laboratory detection, 1, 2, 4, 8, 12, 16, 20, and 24 weeks after inoculation (four leaves from each of six replicates). These samples were sent by overnight courier to the California State Department of Food and Agriculture Diagnostic laboratory (Sacramento, California) where the most current USDA-APHIS approved methodology was used for detection. Samples were both isolated on PARP semi-selective media and tested by ELISA (Agdia Pathoscreen Kit for Phytophthora [#PSA 92600]). All samples were tested further by either real-time or nested PCR.specific for *Phytophthora ramorum*. (APHIS website: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml .) When available, up to 24 leaf samples per species were removed to evaluate the potential for leaf lesions to produce sporangia and chlamydospores (4 leaves from each of 6 replicates). For each sampled leaf, up to 3 (6 mm diameter) leaf-disks were punched from the margin of the lesions. A total of 6 randomly selected leaf-disks from each replicate were placed in a Petri plate and were flooded with enough deionized water to just cover the leaf disks. Plates were incubated at 20 °C in the dark. Sporangia and chlamydospores on the margin of the leaf-disks was identified by propagule type and counted one week after flooding.

Environmental conditions were monitored on the experimental site with a portable digital weather station (Onset Computer Co.). The average maximum, minimum, mean, temperatures and cumulative rainfall were for *summer*: 25.7 °C, 8.0 °C, 15.1 °C, and 1.9 mm respectively; for *fall*: 15.3 °C, 2.8 °C, 8.0 °C, and 257.7 mm respectively; for *winter*: 15.9 °C, 3.9 °C, 9.1 °C, and 235.4 mm respectively; for *spring*: 27.3 °C, 9.6 °C, 17.5 °C, and 5.9 mm respectively. Spring 2006 was notable for its record-breaking high temperatures and low rainfall.

Results and Discussion

Growth rates for camellia and rhododendron lesions were fastest in the fall and winter and slowest in the spring when temperatures were unusually hot (fig. 1 and 2). Leaf lesions developed as “waves” of alternating dark and tan necrotic areas. Dark brown portions surrounded by lighter halos were associated with periods when lesions were expanding rapidly during cool, wet conditions. Light tan areas were associated with periods when lesion growth slowed or stopped during hot and dry conditions. Camellia leaf abscission occurred as a result of *P. ramorum* infection. Leaf abscission occurred more quickly when lesion growth was rapid in the fall and winter. All camellia leaves had fallen by 12 weeks after inoculation. Leaf abscission occurred very slowly in the spring when lesion growth was slow. Most leaves had not abscised for up to 16 weeks after inoculation (fig. 3). Leaves often fell before the lesions became larger than 4 cm^2. Rhododendron leaves abscised infrequently and then only in the winter.

Under laboratory conditions, sporangia and chlamydospore production declined as lesions aged. Flooded camellia leaf-disks were capable of producing chlamydospores and sporangia 8 to 12 weeks after initial inoculation. More propagules were produced in camellia from leaves inoculated in fall and winter than in other seasons (fig. 4).

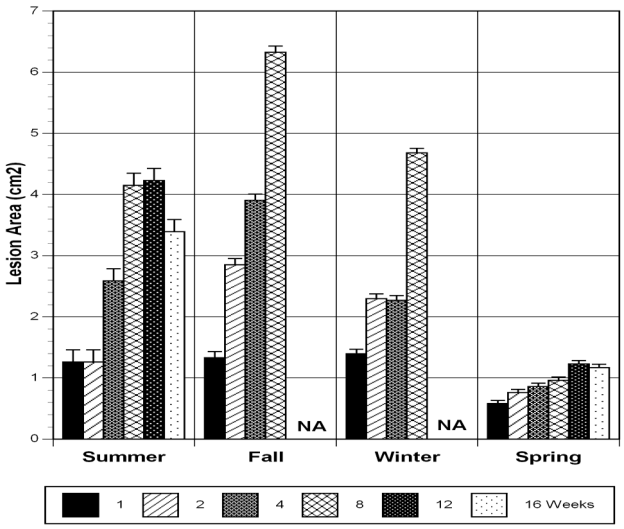

Figure 1—Mean lesion area on camellia leaves (N = 30 at first sampling) inoculated with *Phytophthora ramorum* during different seasons. Different bar patterns correspond to the number of weeks leaves were harvested after inoculation. Error bars indicate the standard error of the mean for each timepoint (NA = not available).

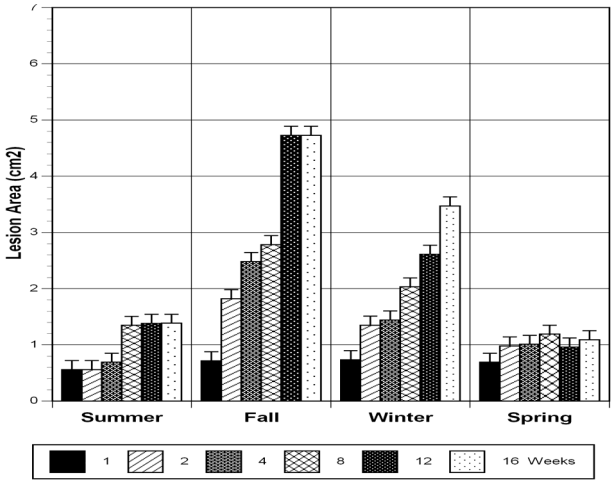

Figure 2—Mean lesion area on rhododendron leaves (N = 30 at first sampling) inoculated with *Phytophthora ramorum* during different seasons. Different bar patterns correspond to the number of weeks leaves were harvested after inoculation. Error bars indicate the standard error of the mean for each timepoint.

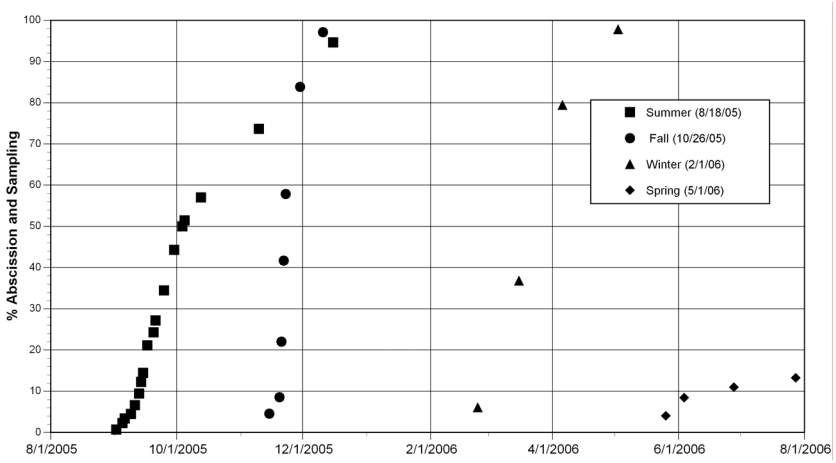

Figure 3—Percentage 420 camellia leaves (N = 420) inoculated with *Phytophthora ramorum* that had abscised or had been sampled after seasonal inoculation.

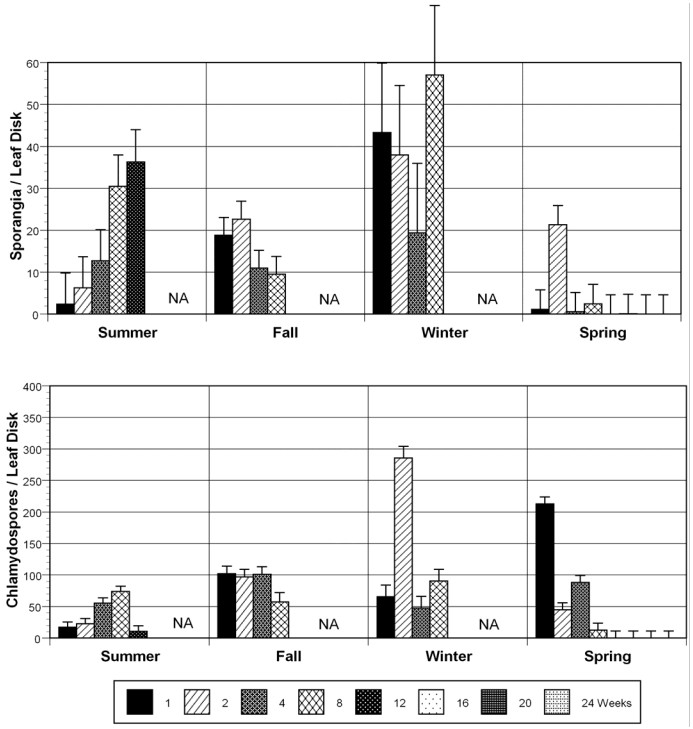

Figure 4—Mean production of sporangia and chlamydospores from *Phytophthora ramorum*-infected camellia leaf disks. Leaves were inoculated once per season and sampled weekly up to 24 weeks after inoculation. (NA = not available.) Upper figure represents sporangial counts, lower figure represents chlamydospore counts. All counts were made from the disk margin of disks flooded with deionized water.

ELISA and PCR (nested and real time) detected *P. ramorum* 100 percent of the time regardless of season and leaf age up to 16 weeks after inoculation. Successful laboratory isolations on PARP media by the state laboratory were made with summer inoculated leaves up to 20 weeks, with fall up to 24 weeks, with winter up to 20 weeks, but with spring only up to 4 weeks after inoculation. Laboratory detection locally (without shipment) with PARP generally mirrored this result (fig. 6).

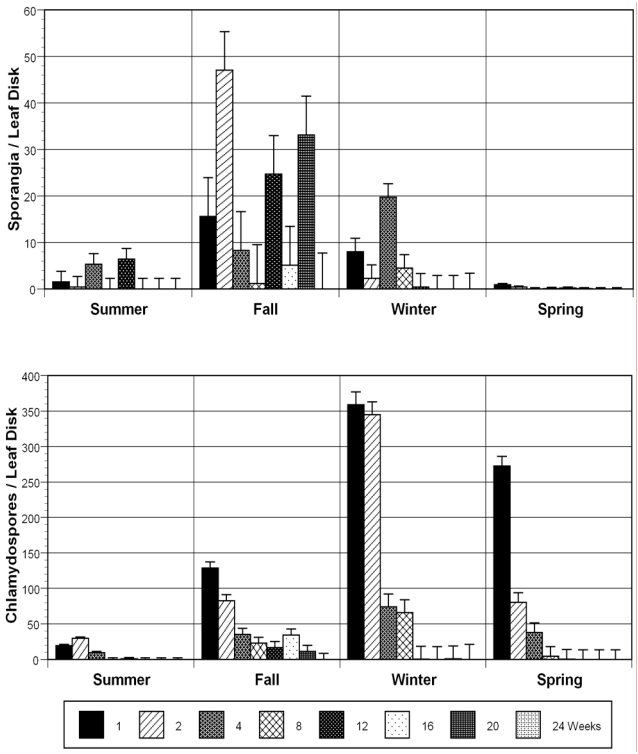

Figure 5—Mean production of sporangia and chlamydospores from *Phytophthora ramorum*-infected rhododendron leaf disks. Leaves were inoculated once per season and sampled weekly for up to 24 weeks after inoculation. (NA = not available.) Upper figure represents sporangial counts, lower figure represents chlamydospore counts. All counts were made from the disk margin of disks flooded with deionized water.

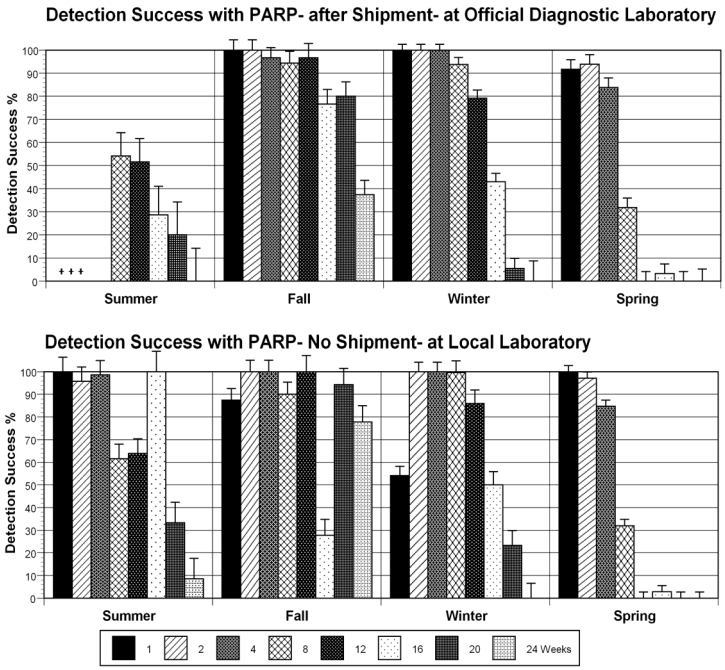

Figure 6—Mean percentage of rhododendron and camellia leaf pieces inoculated with *P. ramorum* that grew on PARP semi-selective media. Leaves from both species were inoculated once per season and then leaves (N = 24 at first sampling) were sampled once per week for up to 24 weeks after inoculation. Detection is by California Department of Food and Agriculture (CDFA) Diagnostic Laboratory (Sacramento) after courier shipment (top chart), or by local laboratory without shipment (bottom chart). Error bars represent standard error of the mean. (Detection at the first three sampling dates cultured at CDFA (top chart) were not quantified, but were all positive (+) for *P. ramorum*.)

Literature Cited

APHIS Federal Order, Posted 2/28/2007. *Phytophthora ramorum*; Quarantine and Regulations, Document #APHIS-2005-0102-0001.

Monitoring for *Phytophthora ramorum* and Other Species of *Phytophthora* in Nurseries and Urban Areas in the Southeastern USA[1]

Yeshi A. Wamishe,[2] Steven N. Jeffers,[3] and Jaesoon Hwang[2]

Abstract

Nurseries in the southeastern United States that received ornamental plants in 2004 colonized by *Phytophthora ramorum* and the surrounding urban areas are being monitored to determine if this pathogen has escaped and become established. At the same time, the prevalence and diversity of other species of *Phytophthora* are being investigated. Water and field soil were collected from six retail nurseries in Florida in February and March 2006 and from three retail nurseries in South Carolina in both spring and fall 2006. Water samples (1 to 2 liter) were collected from streams, retention basins, and irrigation ponds; samples of field soil (1 to 2 liter) were collected from areas where diseased plants previously had been located. In addition, 20 suburban streams draining urban landscapes in South Carolina, where infested or infected plants may have been planted, also were monitored. Streams fed by multiple feeder creeks and that drained large landscape areas were selected for monitoring. Three to seven streams were sampled in each of five cities (Seneca, Greenville, Spartanburg, Columbia, and Aiken) in the central to northern part of South Carolina. A 1- to 2-liter sample of water was collected from each stream in spring and fall 2006. Water samples from nurseries and suburban streams were held in a cool ice chest and processed within 10 to 14 hours after collection. For each water sample, eight subsamples (50 to 200 ml, depending on water quality) were pulled through 47 mm-diameter membrane filters (Nuclepore with 3 μm pores or Durapore with 5 μm pores) by vacuum to trap propagules of *Phytophthora* spp. Filters then were inverted onto PARPH-V8 selective medium to recover isolates of *Phytophthora* spp. Colonies of *Phytophthora* spp. were counted and representative isolates were sub-cultured and stored at 15°C in the dark. For each nursery soil sample, three 100-ml subsamples were flooded with 200 ml of distilled water and camellia and rhododendron leaf pieces were floated on the water surface for 3 days at 20°C in the dark. Leaf pieces then were embedded in PARPH-V8 selective medium, and plates were placed in the dark at 20°C for 7 to 10 days to isolate *Phytophthora* spp. Representative colonies were subcultured and stored as mentioned above. GPS coordinates were recorded at each soil and water sample site, and water temperature and pH were measured at each water site.

At the nurseries in both Florida and South Carolina, *Phytophthora* spp. (including *P. cinnamomi*, *P. citricola*, *P. palmivora*, *P. gonapodyides*, and several unidentified species) were recovered from 13 out of 15 water samples and from 16 out of 24 soil samples. At one nursery in Florida, low levels of *P. ramorum* were recovered from a sample of standing water in a retention basin and from one field soil sample that was collected where infested plants previously had been loaded and unloaded. However, state personnel found camellia plants with ramorum blight nearby when we were collecting our water and soil samples. *P. ramorum*

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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was not recovered when additional water and soil samples were collected 2 weeks later. Therefore, these results may indicate that the *P. ramorum* propagules detected in the two soil and water samples were only transient inocula from the diseased plants present at the time samples were collected and were not evidence of an established population. Additional sampling and testing will be needed to determine whether or not *P. ramorum* is persisting in the area. To date, *P. ramorum* has not been detected in any of the streams draining urban landscapes in South Carolina; however, other species of *Phytophthora* were found in all 20 of the suburban streams sampled. Identification of these species is in progress. *P. gonapodyides* was recovered from all the streams, and the diversity of species of *Phytophthora* appeared to be greater in the fall than the spring. Monitoring of nurseries and suburban streams will continue in 2007.

Key words: Sudden oak death, *Phytophthora* spp. in streams and nurseries, diversity.

Acknowledgments

The authors thank: Florida and South Carolina nursery personnel and Florida Department of Agriculture and Consumer Services personnel in Florida for their cooperation and the Center for Plant Health Science and Technology (United States Department of Agriculture-Animal and Plant Health Inspection Service, Plant Protection and Quarantine) for support and funding.

Four Years Experience With Filtration Systems in Commercial Nurseries for Eliminating *Phytophthora* Species From Recirculation Water[1]

T. Ufer,[2] M. Posner,[3] H.-P. Wessels,[4] S. Wagner,[2] K. Kaminski,[2] T. Brand,[5] and S. Werres[2]

Abstract

In a four year project, three different filtration systems were tested under commercial nursery conditions to eliminate *Phytophthora* spp. from irrigation water. Five nurseries were involved in the project. Slow sand filtration systems were tested in three nurseries. In the fourth nursery, a filtration system with lava grains (Shieer® Bio filtration) was tested and in the fifth nursery, a constructed wetland was investigated. The average filtration capacities per year (2003 until 2005) were between 30,000 and 100,000 m³ water for the three sand filtration systems, about 46,000 m³ for the lava grain filtration system and approximately 5,000 m³ for the constructed wetland filtration system.

In total, between eight (constructed wetland) and 11 (the other nurseries) water samples were taken in May, August and October from 2003 until 2006 before and after filtration and were tested for the occurrence of *Phytophthora* spp. in the laboratory.

Preliminary results from 2003 until August 2006 indicate that the frequency of water samples with *Phytophthora* in the three nurseries testing the slow sand filtration was between 36 percent and 91 percent. All three sand filtration systems eliminated *Phytophthora* completely; in none of the samples taken just after filtration were these microorganisms detected. In the nursery with the lava grain filtration system, about 55 percent of the pre-filtered water samples tested positive for *Phytophthora*. *Phytophthora* was detected in only one sample after filtration. This sample was taken after the filtration system was switched off. At the constructed wetland, about 89 percent of the water samples taken from the water reservoir were contaminated with *Phytophthora* spp. After filtration about 37 percent of the samples were still contaminated. The slow sand filters have lower construction costs, annual costs and costs per m^3 filtered water but have lower volume filtration capacities than the lava grain filter system (Shieer® Bio Filter).

Key words: *Phytophthora ramorum*, sudden oak death, ramorum blight, water, nursery, control, filtration systems.

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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Soil Treatments for the Elimination of *Phytophthora ramorum* From Nursery Beds: Current Knowledge From the Laboratory and the Field[1]

L.E. Yakabe[2] and J.D. MacDonald[2]

Abstract

Over the past years, ramorum blight, caused by *Phytophthora ramorum*, has reoccurred at specific nurseries. In many cases, the re-emergence of the disease could not be traced to a second introduction. Since it is known that *P. ramorum* propagules can survive for over a year in soil, it is not unreasonable to hypothesize re-emergence of the disease may be attributed to inoculum surviving in soil beds. Although the United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) Confirmed Nursery Protocol recommends soil beds be treated with heat, chloropicrin, dazomet, metam-sodium, or methyl bromide, the number of re-emergent nurseries suggests failures of these recommendations. Label recommendations may not provide complete eradication. For example, *P. ramorum* was still detectable after dazomet (Basamid®) (158.77 kg/0.40 ha = 350 lb/acre) was applied to an infested nursery site and sealed with a water cap as opposed to a polyethylene tarp. Additionally, some of the recommended methods are not feasible in many nursery settings due to lack of chemical availability, buffer zone requirements, or township caps. More needs to be known about treatment materials and methods to provide nurserymen with viable options to manage this disease.

Initial in vitro testing, using artificially-infested soil in mason jars, has been conducted to test various rates, combinations, and application methods of (1) chloropicrin, (2) 1,3-dichloropropene, (3) 1,3.dicholopropene with 35 percent of chloropicrin (Telone C35®), (4) metam sodium (Vapam®), (5) iodomethane, (6) dazomet (Basamid®), (7) dimethyldisulfite, (8) hydrogen dioxide (Terraclean®), and (9) hydrogen dioxide (Zerotol®), using a polyethylene barrier to prevent escape of volatiles where applicable. Chemicals were tested at recommended, two times recommended, and half recommended rates. Treatments were applied at the position in the soil profile recommended or at the top of the soil profile. Treated soil was covered with polyethylene for two weeks or the label minimum re-entry period. Except for dimethyldisulfite, 1,3-dichloropropene, Terraclean®, and Zerotol®, *P. ramorum* was not detectable after treatments under *in vitro* conditions. Dimethyldisulfite (90.72 kg/0.40 ha = 200 lb/acre), 1,3-dichloropropene (45.36 kg/0.40 ha = 100 lb/acre), Terraclean® (1:1000 dilution applied to saturation) and Zerotol® (1:50 dilution applied to saturation) only reduced the number of viable propagules.

In 2005, three cooperating ornamental nurseries with infested sites suitable for fumigation opted to use Basamid® (158.76 kg/0.4. ha = 350 lb/acre) incorporated throughout the soil profile. The beds were sealed with a polyethylene tarp for 14 days. *Phytophthora ramorum* was not detected after treatment. Two of these nurseries have not been positive in 2006. One

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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nursery was positive in 2006, but the infested bed had no relation to the previous 2005 treated plot.

In 2005 and 2006, other nurseries that could not apply fumigants opted to treat their infested soil with hypochlorite, quaternary ammonia, or phosphites. In each case *P. ramorum* was detected after these treatments. To provide treatment options for nurseries unable to fumigate, we have begun experiments utilizing solarization and steam treatments.

Recommended Industry Best Management Practices for the Prevention of *Phytophthora ramorum* Introduction in Nursery Operations[1]

Karen Suslow[2]

Abstract

The following industry recommended best management practices (BMPs), designed for growers and/or interstate shippers of host and associated host plants of *Phytophthora ramorum*, consists of biosecurity guidelines created by and for nursery growers in order to reduce the risks associated with *P. ramorum*. The control of *P. ramorum* is based on the insertion of multiple hurdles to prevent the introduction of the pathogen into nursery operations.

The BMPs were created in 2002 when the first United States Department of Agriculture (USDA) *P. ramorum* compliance agreements were issued to interstate shippers from California (CA) quarantined counties. Over the past several years, the draft document has been reviewed and input provided by Agricultural Research Service (ARS) scientists, nursery people, National Plant Board (NPB) members and Horticultural Research Institute members to reflect advancements in research on this pathogen. Collaboration efforts were also undertaken with Canadian and British regulatory agencies.

The BMPs are divided into four management categories: Pest Prevention/Management, Training, Internal/External Audits, Records/Traceability and Documentation. Individual nurseries are encouraged to review these practices and voluntarily apply some or all of them, depending upon their production systems, physical location, nursery type, regional climatic conditions, geographical location and the plants grown. The document is a draft and will continually be updated as research is conducted and made available.

A pilot program to determine the benefit of the BMPs is being developed with the three western state nursery industries and state regulatory agencies operating as third-party auditors.

Key words: *Phytophthora ramorum*, best management practices, prevention, nursery operations.

Best Management Practices for Nurseries

The following industry recommended best management practices (BMPs), designed for growers and/or interstate shippers of host and associated host plants of *P. ramorum*, consists of biosecurity guidelines created to assist nursery crop producers in developing an effective monitoring and action plan to reduce the risks associated

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5-9, 2007, Santa Rosa, California.

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with *P. ramorum*. The control of *P. ramorum* is based on minimizing the risk of introduction and preventing the survival of the pathogen within the nursery.

This draft document is designed to offer scientifically-based risk mitigation measures for nurseries. Individual nurseries are encouraged to review these practices and voluntarily apply some or all of them, depending upon their production systems, physical location, nursery type, regional climatic conditions, geographical location and the plants grown.

In the future, it is envisioned that a formal BMP program may be implemented as an alternative to the current federal regulatory approach. Such a program would likely be established as a federal program with collaboration of the United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS), the nursery industry, and state departments of agriculture. In a formal program context, each BMP would be accompanied by a documentation sheet identifying the practice that is employed. If a participating grower, in collaboration with the appropriate auditor or oversight authority, determines the BMP is appropriate for the specific nursery, the Site Specific 'Box' would be checked. Additionally, if a BMP is not applicable for a particular site, the N/A 'Box' would be checked. The BMPs which are equivalent to requirements currently mandated in the *P. ramorum* Federal Interim Rule, 7 CFR 301.92, are noted as such in the left margin next to each BMP in this document. Each of the BMPs marked "Regulated" are mandatory.

The 'auditor' or oversight authority would review the documentation sheets on an annual basis. Nurseries will also be responsible for notifying the auditor or oversight authority when a modification to a nursery practice occurs.

The following recommended best management practices for nurseries "free from" *P. ramorum* were developed using the North American Plant Protection Organization's Regional Standard for Phytosanitary Measures (RSPM) Number 24. These risk mitigation measures may be utilized as a stepping stone to a clean stock-like program, such as the United States nursery certification program.

I. Pest Prevention/Management

- A. Moisture Management
- B. Nursery Layout
- C. Cleaning and Sanitation/Plant Debris Handling and Disposal
- D. Weed Control and Established Nursery Plants

II. Training

III. Internal/External Monitoring/Audits

- A. Inspection of Plants

IV. Records/Traceability

- A. Incoming Plants and Returned/Returning Plants
- B. Record Keeping

V. Documentation of Program Procedures

VI. High Risk Plants

I. Pest Prevention/Management

A. Moisture Management:

GOAL: Minimize moisture conditions condusive to *P. ramorum.*

Regulated | Specific to Nursery | NA

BMPs to consider implementing to reach stated goal:

☐ ☐ ☐ **1) Avoid overhead irrigation of high-risk plants. Irrigate in a manner to avoid prolonged leaf wetness of 12 hours or more.**

Rationale:
Properly time irrigation events to reduce conditions favorable for disease development. Extended leaf wetness (such as overnight) is conducive to pathogen infection.

Requirement for External Audit: documentation of irrigation practices

☐ ☐ ☐ **2) Irrigation water from any source other than well or municipal water supplies shall be monitored and tested to confirm that it is free from the pathogen.**

Rationale:
For growing operations that utilize open irrigation water sources (ponds, lakes, streams), and/or who blend both well and surface water sources for irrigation purposes, proper water treatment (in other words ozonation, chlorination or other water disinfection program) is recommended. Attention also needs to be directed to possible well water contamination with the *P. ramorum* pathogen by back siphoning of irrigation water or water/soil into the system.

Requirement for External Audit: documentation of water sources

☐ ☐ ☐ **3) Divert soil and water movement, during storm-related events, from hillsides populated with *P. ramorum* host plants.**

Rationale:
Keep possible offsite contamination from entering production location. Unless the offsite area has been properly surveyed and determined to be *P. ramorum* free, the grower cannot assume that run-off from off-site is not contaminated with *P. ramorum* spores.

Requirement for External Audit: nursery site inspection

☐ ☐ ☐ **4) Avoid or minimize accumulation of standing surface water in containerized high-risk (HR) plant beds.**

Rationale:
Phytophthora spp. are transmitted via water and repeat finds occur more often in HR plant beds where standing water accumulates. The pathogen may potentially enter through the roots or by splashing onto leaf surfaces.

Requirement for External Audit: documentation of irrigation practices

B. Nursery Layout:

GOAL: Reduce potential introduction and minimize the spread of *P. ramorum* through nursery operations.

BMPs to consider implementing to reach stated goal:

Regulat Specific to Nursery

☐ ☐ ☐ **1) Reduce potential inoculum splash from high-risk (HR) plants to other crops.**

a. Create a physical barrier between HR plants and all other crops or
b. Create a 2-meter break between HR plants and all other crops or
c. Develop preventive spray program year round when plants haveleaves or
d. Interplant with non-host plants to the genus level.

Rationale:
Many positive plants have been associated with nurseries that have also had positive camellias and/or rhododendrons. (See High Risk proposal)

Requirement for External Audit: nursery site inspection

☐ ☐ ☐ **2) Review your Field Layout Plan. Determine how you can minimize the impact of the USDA Confirmed Nursery Protocol if *P. ramorum* is found. Break up long sections of host and associated host plants (HAP) with non-HAP material to the genus level. (USDA-Agricultural Research Service is investigating potential non-host material.)**

Rationale:
Nursery production bed layout, mixing or alternating of HAP and non HAP plant material in production beds can help eliminate large contiguous monocultures of plants that are *P. ramorum* susceptible.

Requirement for External Audit: mapping of stock location

☐ ☐ ☐ **3) Maintain a separate cull pile for high-risk plants so it is not included in the soil recycling pile for potential future reuse. If infested plants are found, the pile must be quarantined and treated, or disposed of, according to regulatory requirements.**

Rationale:
Proper sanitation measures reduces the risk of spreading the pathogen in the recycled soil within and outside the nursery.

Requirement for External Audit: nursery site inspection

C. Cleaning and Sanitation/Plant Debris Handling and Disposal:

GOAL: Reduce potential introduction and minimize the spread of *P. ramorum* through nursery practices.

BMPs to consider implementing to reach stated goal:

Regulated | Specific to Nursery | NA

☐ ☐ ☐ **1) For nurseries in high risk (HR) areas (near a native find) or for recurrent nurseries, pick up and dispose of leaf debris in HR plant production areas during the time of year when the pathogen is most prevalent or institute a preventative spray program.**

Rationale:
General sanitation practice. Use of a leaf vacuum is an appropriate method to gather leaves during the time of year when the pathogen is most prevalent, for example early and late winter. Proper disposal of leafy debris should be governed by appropriate local/state/federal recommendations (bagging, burning, burying off site, and so forth).

Requirement for External Audit: nursery site inspection.

☐ ☐ ☐ **2) After every crop rotation, disinfect propagation mist beds, sorting area, cutting benches, machines and tools to minimize the spread or introduction of pathogens.**

Rationale:
Basic sanitation practices should be followed using registered fungicides in accordance with label instructions to reduce possible points of entry/contamination in the production cycle.

Requirement for External Audit: documentation of nursery personnel training

☐☐☐ **3) If you visit known *P. ramorum* infested areas, wash shoes, tools and vehicles that may have contacted contaminated soils before traveling to disease free areas.**

Rationale:
Best defense is to not visit areas where known infestations are occurring to reduce possible accidental introduction of the pathogen into the nursery production site. If grower has visited infested areas, appropriate sanitation measures (washing and steam cleaning of trucks, and so forth) as recommended by regulatory authorities should be undertaken.

Requirement for External Audit: documentation of nursery sanitation procedures training

☐☐☐ **4) Use new or clean and sanitized pots for high-risk plant production.**

Rationale:
This measure reduces the potential of any unknown residual *P. ramorum* contamination on the nursery site and possible further disseminating of the pathogen throughout the nursery. New pots should be stored and handled in such a manner as to avoid contact with potential *P. ramorum* sources. Recycled pots should be thoroughly cleaned of any residual substrate and disinfected before reuse. Recycled pots should also be stored and handled in such a manner as to avoid contact with potential *P. ramorum* sources.

Requirement for External Audit: documentation of nursery sanitation practices

☐☐☐ **5) Ensure runoff from all cull piles is directed away from soil components, soil mixing area, and growing beds to prevent contamination.**

Rationale:
Avoids any possibility of cross contamination. If growers cull infested material, sanitation methods should be established to clean/disinfect trucks, wagons, and tools that are used to move infested material.

Requirement for External Audit: nursery site inspection

☐☐☐ **6) For plants that are prone to disease, chemically treat crop in field prior to taking cuttings and dip cuttings in an approved disinfectant solution before sticking.**

Rationale:
Treatment of stock plants with registered disinfectant(s) before cutting of the propagation material can reduce the

possible introduction of contaminated plant material into the propagation cycle and protect the open wounds from possible pathogen infection.

Requirement for External Audit: nursery pesticide

D. Weed Control and Established Nursery Plants:

GOAL: Reduce the potential for inoculum buildup of *P. ramorum* in weeds and established nursery plants**.**

Regulated | Specific to Nursery | NA

BMPs to consider implementing to reach stated goal:

☐ ☐ ☐ **1) Manage weeds on the nursery site as they can potentially serve as alternate hosts of *P. ramorum*.**

Rationale:
Maintaining clean cultivation in and around the production site may eliminate possible reservoirs of *P. ramorum* pathogen. Since it is not known if insect vectors can also carry *P. ramorum*, clean cultivation will reduce opportunities for insect infestations and contamination in the nursery.

Requirement for External Audit: nursery site inspection

☐ ☐ ☐ **2) No over story or under story of known *P. ramorum* hosts on nursery growing grounds should be maintained unless regular monitoring of those hosts is performed.**

Rationale:
Reduce the potential of offsite contamination of *P. ramorum* into the production site by establishing a regular monitoring program for *P. ramorum* host plants in the environs of the nursery. Monitoring program should be based upon the specific life cycle of the disease within that specific growing region and the time of year when the pathogen is most prevalent.

Requirement for External Audit: nursery site inspection

II. Training

GOAL: Enhance prompt disease recognition.

Regulated | Specific to Nursery | NA

BMPs to consider implementing to reach stated goal:

☒ ☐ ☐ **1) Nursery personnel should attend one or more *P. ramorum* trainings conducted by qualified personnel or document self-training via one of the two websites below**

Rationale:
Responsibility for *P. ramorum* management on nursery site should be the responsibility of a specified group of trained nursery personnel. These individuals should be trained in all aspects of the management of the disease. Special attention should be given to staying informed of new research findings regarding the disease and any changes in regulations regarding plant sampling, testing or shipping of product. Training is available through the USDA-Forest Service, CA Oak Mortality Task Force (COMTF), state agriculture departments, county agricultural commissioners offices or through selected universities.

On line at USDA website:
www.aphis.usda.gov/ppq/ispm/pramorum
Or on line at COMTF website: www.suddenoakdeath.org

Requirement for External Audit: documentation of training

☒ ☐ ☐ **2) Educate nursery personnel to recognize and report pest or disease problems.**

Rationale:
Personnel should be trained to not only look for *P. ramorum* symptoms but for any symptoms of plant abnormality in the production system.

Requirement for External Audit: documentation of training

☒ ☐ ☐ **3) Educate employees and managers about their company's implemented BMP's.**

Rationale:
Appropriate manager(s) should work with their state agriculture department, county agriculture department and/or knowledgeable university personnel to identify the specific, appropriate recommended BMPs to implement in order to minimize the risk of introduction of *P. ramorum* into their nursery operation.

Requirement for External Audit: documentation of training

III. Internal and External Monitoring/Audits

GOAL: Regularly inspect plants in and around the nursery to ensure earliest possible detection of *P. ramorum* infection.

Regulated NA

BMPs to consider implementing to reach stated goal:

☒ ☐ ☐ **1) Annual nursery inspection of all plants in the nursery with a focus on *P. ramorum*-like symptoms. Inspection includes mandatory testing of at least 40 symptomatic samples.**

Rationale:
The host list continues to expand and as a result, all plants need to be inspected for *P. ramorum*-like symptoms. Current state and federal regulations require a minimum of 40 samples to be taken and tested.

Requirement for External Audit: annual nursery inspection report

☒ ☐ ☐ **2) Nursery to inspect high-risk (HR) plants, such as camellias and rhododendrons, monthly throughout the growing season, particularly after pruning or a significant weather event.**

Regulators to inspect HR plants twice a year. Train employees to look for and report symptoms when working with the HR plants.

Rationale:
Camellia and *Rhododendron* species have comprised the majority of the total positive plants in nursery settings throughout the regulated area. (See High Risk Proposal- under discussion if to whether or not this BMP should be regulated)

Requirement for External Audit: documentation of nursery practices

☐ ☐ ☐ **3) Routinely monitor incoming HAP for symptoms of *P. ramorum.***

Rationale:
First line of defense. Grower priority should be to ensure that potentially contaminated stock is not allowed to enter the production site.

Requirement for External Audit: documentation of nursery practices

☐ ☐ ☐ **4) Routinely* inspect HAP in the landscape on the growing grounds and in the surrounding area for symptoms of *P. ramorum*.**

Rationale:
HAP plant material should be visually screened on a regular basis for any abnormalities. *Special attention should be given to those times when the pathogen is most prevalent.

Requirement for External Audit: documentation of nursery practices

☐ ☐ ☐ **5) Ensure the use of *P. ramorum* free growing media/growth substrate.**

Rationale:
Given that *P. ramorum* may contaminate potting substrates, it is critical for the grower to reduce any sources of contamination in peat, bark, and other organic components of the substrate. Proper documentation of disease free substrate materials shipped into the site should be obtained. Proper storage and prompt use of substrate materials (for example covered, prevented from contact with native soil), is critical.

Requirement for External Audit: documentation of nursery practices

IV. Records/Traceability

A. Incoming Plants and Returned/Returning Plants:

Regulated | Specific to Nursery | NA

GOAL: Reduce the potential introduction and spread of *P. ramorum* through nursery trade.

BMPs to consider implementing to reach stated goal:

☒ ☐ ☐ **1) Confirm nursery stock is propagated from materials obtained onsite, or is received from nurseries that are licensed and/or certified according to all applicable phytosanitary laws and regulations.**

Rationale:
First line of defense. Know your supplier. Grower priority should be to ensure that potentially contaminated stock is not purchased or allowed to enter production site.

Requirement for External Audit: documentation of nursery practices

☐☐☐ **2) Avoid product returns of nursery stock from a receiver in a quarantined area or from nurseries that are not under *P. ramorum* compliance. If unavoidable, contact your county agriculture department or appropriate plant regulatory agency prior to accepting the nursery stock return.**

Rationale:
Avoids possible cross contamination. Returned stock may have been exposed to *P. ramorum* prior to return.

Requirement for External Audit: nursery map, documentation of nursery practices**.**

☐☐☐ **3) Nurseries should avoid commingling incoming HAP nursery stock with existing stock.**

Rationale:
Avoids cross contamination of clean and potentially diseased material. Assists with inventory control and tracking of plant material in the nursery.

Requirement for External Audit: documentation of nursery practices, nursery site inspection

☐☐☐ **4) For HAP buy-ins, suspend the use of *Phytophthora* specific fungicides on 10 percent or 100 plants, whichever is fewer, for a two month period to determine if fungicides that may have been used by seller were masking symptoms of *P. ramorum* or, through your state agricultural department, sample and test a representative group via ELISA or PCR. If tests are negative, the above BMP is not applicable.**

Rationale:
This recommendation correlates with a3 (above) and supplements isolation efforts.

Requirement for External Audit: documentation of nursery practices

☐☐☐ **5) Authorized and knowledgeable personnel should visually inspect all nursery stock (buy-ins, transfers, and returns), regardless of origin, for symptoms of *P. ramorum* prior to introduction into the nursery facility.**

Rationale:
Because not all areas of the country can be certified *P. ramorum* free, this visual evaluation of off-site nursery stock can provide a major screening defense to the introduction of the pathogen.

Requirement for External Audit: documentation of nursery personnel training, documentation of nursery practices

☐ ☐ ☐ **6) Off load incoming HR plant shipments to an area that can be cleaned of leafy debris. Sweep incoming plant debris from the receiving area and the delivery truck. Collect debris and dispose of appropriately.**

Rationale:
Basic sanitation to remove possible sources of disease inoculum. Proper disposal of leafy debris should be governed by appropriate local/state/federal recommendations (bagging, burning, burying off site, and so forth). Composting of infected plant debris is not an acceptable practice. Leaf litter has been shown to be a potential source of inoculum.

Requirement for External Audit: documentation of nursery

☐ ☐ ☐ **7) Monitor sanitation practices of delivery trucks that ship HR plants. Assure that trucks are properly cleaned of plant debris between shipments.**

Rationale:
Trucks may be a source of inoculum if not cleaned properly.

Requirement for External Audit: documentation of nursery practices

B. Record Keeping:

GOAL: Keep incoming and outgoing plant records for the purpose of identifying where plants originated and where plants have been send in the event the nursery is found infested with *P. ramorum*.

Regulated | Specific to Nursery | NA

BMPs to consider implementing to reach stated goal:

☒ ☐ ☐ **1) Maintain for two years minimum: accurate shipping documentation identifying product, amount, date and origin or receiver for the purpose of identifying trace backs and trace forwards.**

Rationale:
Proper documentation protects not only the grower but also the receiver of plant material. Production operation should investigate methods for quick recording and retrieval of documentation. Disease monitoring and scouting results should be integrated with inventory control to provide rapid trace forward and back of suspected infested nursery stock.

Requirement for External Audit: nursery inspection (of records).

☐☐☐ **2) Consider strategies that would facilitate the rapid identification and segregation of product based upon production location from the time it has left the growing operation through final sale.**

Rationale:
Operations personnel should develop a "Code Red" crisis management plan for dealing with possible *P. ramorum* infestations that stresses containment and considers all aspects of the plant production cycle, but especially the movement of plant material around site and shipping off site.

Requirement for External Audit: written nursery "Code Red" plan

V. Documentation of Program Procedures

GOAL: Provide proof that the nursery's BMP's are documents and implemented.

Example information to include in manual:

1) Employee training records
2) Internal systems review procedure
3) List of implemented BMPs that are appropriate for your site based upon the nurseries specific production systems, physical location, nursery type, regional climatic conditions and the plants grown. Documentation sheets are being developed for each BMP

VI. High risk plants

The NPB Western region high risk proposal is aimed at camellias and rhododendrons with the caveat that should any other plant demonstrate the same level of risk, they be added to the mitigation measures the Working Group agrees to. In the western region, 88 percent of infected plants are in either of these two genera.

1) In CA: *Camellia* and *Rhododendron*
2) In BC, OR, WA: *Camellia*, *Rhododendron*

This document is a work in progress. Please use the following contact information for all questions, comments or suggestions:
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Forest Insects and Pathogens: Quarantine Issues

Sequoia sempervirens

Facts or Friction: The Evolving Role of Science in Phytosanitary Issues[1]

Eric Allen[2]

Abstract

With the expansion of global trade, problems with invasive alien pests have also grown. In order to reduce the international movement of plant pests and protect valuable plant resources, national plant protection regulations and international standards continue to be developed. Science is critical to the development of effective national and international plant protection regulations aimed at reducing the spread of plant pests. There is an increasing recognition that such regulations be "science-based" as identified in the World Trade Organization Sanitary and Phytosanitary Measures (WTO-SPS) agreement. This need is clearly recognized by the Commission on Phytosanitary Measures (CPM), the governing body of the International Plant Protection Convention (IPPC). The CPM has established expert working groups and technical panels with scientific capacity to support the development of international phytosanitary standards. Science is valuable to plant health regulators as it is a useful tool to identify and address plant pest problems, and is often used in "technical justification" required in domestic and international trade disputes.

Within the structure of the IPPC, a group known as the Standards Committee initiates new international plant protection standards, relying on expert working groups and technical panels to provide accurate scientific information as a basis for their development. Members of these groups are recognized global experts in science and plant health regulatory issues and work from within their national institutions (universities, government agencies, etc) for the benefit of the IPPC. Another group, the International Forestry Quarantine Research Group (IFQRG) is an independent body formed in 2004 focusing on global forestry quarantine issues. This group of scientific specialists and plant health regulators produces issue-specific analyses and undertakes collaborative research in cooperation with the IPPC.

Although much of this work is carried out in a spirit of cooperation, trade considerations and an increasing global awareness regarding phytosanitary issues can lead to disagreements and scientific information is often requested to help resolve disputes. However, there is often a shortage of published information and disputing countries may have different interpretations of experimental results. In recognition of these challenges, there are global efforts to increase international research collaborations to address existing and anticipated phytosanitary problems, where possible standardizing research protocols with cooperative analyses of results. It is hoped that this will lead to greater clarification of pest problems, improved regulations to minimize pest movement, and a reduction in pest-related disputes.

Key words: Regulatory, phytosanitary, International Plant Protection Convention.

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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Phytophthora ramorum + P. kernoviae = International Biosecurity Failure[1]

Clive Brasier[2]

Key words: *Phytophthora ramorum*, *P. kernoviae*, biosecurity, plant health.

Introduction

For a scientist, my title may seem a little sensationalist in tone. This is deliberate - to draw attention to my issue. And here's the issue. About six years ago the previously unknown invasive pathogen *P. ramorum* sp. nov. was found spreading on trees and shrubs in North America and Europe. Almost simultaneously in the U.K. we found another previously unknown invasive *Phytophthora* on trees and shrubs, *P. kernoviae* sp. nov. Shortly before that we found the previously unknown invasive *P. alni* sp. nov. spreading on alders; and a decade before that the invasive *P. ilicis* on holly… Four invasive *Phytophthora* species, each spreading for some years prior to their discovery. And that's just the *Phytophthora* species.… And that's just the invasive *Phytophthora* species we've actually found.

As scientists we usually discuss sudden oak death in the context of our specialist scientific disciplines such as epidemiology, evolutionary biology, fire control, diagnostics and so on. But the bottom line is that the 'sudden' appearance of a *P. ramorum,* a *P. kernoviae,* or a *P. alni* is a symptom of a far bigger underlying problem: the growing threat to our forests and natural ecosystems from invasive forest pathogens (consult Brasier.and others 2006). Or, to put it another way, the low efficacy of current international plant biosecurity protocols. I suggest that this is the *main* scientific issue that we face. *P. ramorum, P. kernoviae, P. alni, P. ilicis* are merely symptoms of the problem.

At the first sudden oak death symposium at Monterey in December 2002 the author presented a scientific health check on the international protocols governing plant movement. I concluded that although the international system was well regulated in many countries, for example North America and the U.K., it could not succeed in protecting our forests and natural ecosystems because of certain fundamental flaws (summarised in Brasier 2005). One proposed flaw was that many invasive pathogens were unknown to science until they escaped from their natural range, in other words, until they became an invasive. There may be many such unknowns (90 percent of all fungi? consult Hawksworth 2001). Such unknown threats cannot be formally regulated against because they do not meet the requirement of being 'listable' as a named threat organism. Before they were discovered causing damaging diseases in the U.S. and Europe both *P. ramorum* and *P. kernoviae* were unknown to science. Obviously, it was by then too late to prevent their initial escape. Both

[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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Phytophthora species probably arrived on plants imported by the nursery trade or by plant collectors.

As with most new invasives, with the discovery of *P. ramorum* and *P. kernoviae* we have been caught napping and left scrambling for scientific information. In this talk I am going to pick out some of the excellent scientific information now emerging on the behaviour of *P. ramorum* and *P. kernoviae* and consider what it may be telling us about the efficacy of plant biosecurity.

Genetic Structure of *P. ramorum* and *P. kernoviae* Populations: Near Clonal Molecular Polymorphism

Studies by Ivors and others on AFLP or microsatellite polymorphisms in *P. ramorum* populations of Europe and North America (Ivors and others 2006) and by Hughes and colleagues (K. Hughes, personal communication) on *P. kernoviae* populations in the U.K. are revealing remarkable genetic uniformity within each species, consistent with genetic bottlenecks following introductions. Together with evidence for an initially limited geographic distribution of each species followed by spread out of the initial areas, this genetic evidence is consistent with the recent introduction of *P. ramorum* into the U.S. and Europe and of *P. kernoviae* into the U.K. Both species are considered to be introduced from an exotic or unexplored ecosystem in another part of the globe (see Brasier and others 2004).

This re-enforces the point that the escape of scientifically *unknown* pathogens from exotic ecosystems is what we most need to prevent. Yet, as already indicated, our international plant biosecurity protocols are based on lists of known, named organisms. In reality, unknowns may represent a majority of the threats. In my opinion, international plant biosecurity protocols need changing to take account of this reality. Otherwise, as with *P. ramorum* and *P. kernoviae,* we may be will be forever chasing the ripples after the splash, not preventing the splash (Brasier 2005).

Geographical Origins of *P. ramorum*, *P. kernoviae*

The geographic origins of *P. ramorum* and *P. kernoviae* have been suggested to be in places such as Yunnan or the Himalayas (Brasier and others 2004). The ‘sudden’ appearance of these species in Europe and North America has already spawned two expeditions to search for their origins: a United States Department of Agriculture-Forest Service funded expedition to forests of Yunnan in 2005 involving Ellen Goheen, Everett Hansen and Niklaus Grunwald; and a part FAO/ part self funded expedition to forests of Nepal also in 2005 involving Andrea Vannini, Anna Brown, Anna Maria Vettraino and Clive Brasier. The Yunnan crew isolated *Phytophthora* spp., but unfortunately ran into local difficulties with bringing out their cultures. The Nepal crew was more fortunate. No *P. ramorum* or *P. kernoviae* was found but, in addition to *P. citricola* and a *P. palmivora*-like *Phytophthora,* an apparently previously unknown forest *Phytophthora* species was discovered in the Himalayan sub tropical forest zone.

The probable existence of another ‘new’ forest *Phytophthora* in Nepal again emphasises that it is the scientifically unknown pathogens that we most need to worry

about. Indeed, for a number of reasons we need to foster international cooperation and encourage funding agencies to support more of the above types of expeditionary searches. They represent part of a more proactive approach to providing information for improving international plant biosecurity policy: too much of current research in plant biosecurity is institutionalised and 'box ticking' (Brasier 2005). Such searches can also help identify likely pathways of exotic pathogen arrival. Furthermore, the study of a pathogen in its native habitat can shed light on its natural host range, and ecology and dispersal: and its 'normal' population structure and population dynamics. Indeed the population dynamics of endemic *P. ramorum* may be quite different from invasive *P. ramorum*. Searches in areas where organisms such as *P. ramorum* are indigenous may also lead to discovery of naturally occurring biocontrol agents, and so widen our disease control armoury.

Origins of *P. kernoviae* – Emerging Information

In Cornwall, U.K., *P. kernoviae* is behaving as a recent invasive in terms of its distribution, its spread and its genetic homogeneity. The recent publication of the formal description of *P. kernoviae* (Brasier and others 2005) has led to its being identified from soil at four different field locations in New Zealand (M. Dick and others, New Zealand MAFF/Ensis, personal communications). Circumstantial evidence indicates it has probably been present at one location in New Zealand since at least the 1950s. It is also known that there have been contacts between nurseries and plant collectors in Cornwall and nurseries and plant collectors in New Zealand. However it is still unclear whether *P. kernoviae* is introduced to New Zealand or is native there.

Behavioural Differences Between the EU1 and NA1 Molecular Lineages of *P. ramorum*

Research carried out on both sides of the Atlantic (for example Werres and Kaminski 2005; Huberli and others 2006; Brasier and others 2006) has shown that the two most widespread molecular lineages of *P. ramorum*, one occurring in Europe (termed the EU1 lineage) and the other dominant in North America (the NA1 lineage), differ significantly in continuous phenotypic characters. For example they differ in their colony patterns, in their mean growth rate and in their mean aggressiveness on susceptible hosts; and EU1 isolates are much more stable in culture than NA1 isolates. These differences indicate adaptive differences between the two lineages. In addition the EU1 lineage is of predominantly A2 sexual compatibility type and the NA1 lineage of A1 type.

Since *P. ramorum* is potentially 'heterothallic' in other words, a sexually outcrossing fungus, these differences indicate that genetic recombination between the EU1 (A1) and NA1 (A2) lineages could yield new phenotypes. Even without A1 x A2 sexual mating, genetic recombination might occur via zoospore fusion between the EU1 and NA1 lineages. This emphasises the need to identify and prevent the spread and intermixing of different *genotypes* of invasive pathogens. We cannot concentrate *solely* on preventing the spread of different pathogen species.

Host Range Studies

It is often popularly assumed that the initial pathway of introduction of *P. ramorum* into Europe or North America was via imported *Rhododendron, Camellia,* or *Viburnum* nursery stock; and of *P. kernoviae* via imported *Rhododendron* or *Magnolia* stock. However, experience coming from host records in the field and from laboratory-based host testing could indicate something rather different. The potential host range of both species is clearly very wide: over 100 foliar and tree stem hosts have been recorded in the field in the U.S. and Europe for *P. ramorum*; over 30 in the U.K. for *P. kernoviae*. Laboratory tests reveal an ever widening potential host range for both species. It has also been clear for some time that *Rhododendron* is probably something of a 'universal *Phytophthora* suscept', it apparently being susceptible to most of the *Phytophthora* species that can attack woody hosts. *Rhododendron* may therefore simply be the ideal universal '*Phytophthora* carrier' (consult Brasier and others 2004).

These observations have a range of implications for biosecurity protocols. First, the initial pathway host or a main pathway host in the invaded area, such as rhododendron, may not necessarily be the common host in the pathogen's geographic area of origin. To identify the host(s) in the area of origin the net may therefore have to be thrown very wide. Second, the initial pathway of introduction may not even have involved rhododendron at all but another host altogether, *P. ramorum or P. kernoviae* spreading onto rhododendron subsequently. Third, a *Phytophthora* pathogen listed on quarantine schedules as causing damage on a specific host in country A might be a serious threat to entirely different hosts and ecosystems in country B. The absence of that specific host in country B is therefore insufficient reason to consider the pathogen 'non-threatening'.

Asymptomatic Sporulation on the Host

There have recently been two interesting new developments on the issue of sporulation behaviour. A study by Riedel and colleagues in Germany (Poster, this Proceedings) has shown that *asymptomatic* roots of infected rhododendrons harbour chlamydospores of *P. ramorum*. Similarly studies by Denman, Moralejo and colleagues in U.K. and Spain (this Proceedings) have shown that sporangial production by both *P. ramorum* and *P. kernoviae* occurs on *asymptomatic* infected leaves and fruits of a range of hosts including rhododendron, *Quercus, Rosa, Smilax, Crataegus and Laurus.*

Assessment of the health of planting stock for quarantine certification is normally a visual process. Many shipments of plants for planting are sent bare rooted. The above studies show that visually healthy plants may harbour a sporulating pathogen in the roots or the foliage, in other words, visual inspection alone may be insecure. They also show that shipping stock bare-rooted is not a guarantee of plant biosecurity. Indeed it might provide a false sense of security.

Presence of *P. kernoviae* and *P. ramorum* in Tree Xylem

Historically, tree *Phytophthora* species have not been perceived as inhabitants of secondary xylem but as inhabitants of the phloem and cambium. Studies by Brown and Brasier (2007) in the U.K. and Parke and others (2007) in Oregon have now shown that *P. kernoviae* and *P. ramorum* are significant colonisers of xylem of for

example *Fagus, Quercus, Acer* and *Lithocarpus* species; that *P. ramorum* and *P. kernoviae* may spread within xylem and possibly recolonise the phloem from the xylem; and that *P. ramorum* and *P. kernoviae* can remain viable (perennate) within xylem for two or more years.

From a biosecurity perspective, these studies indicate that removal of outer sapwood should be undertaken in protocols aimed at preventing national or international spread of *P. ramorum*, *P. kernoviae* and other tree stem *Phytophthora* species in affected timber: Brown and Brasier (2007) recommend removal of at least 3 cm of outer sapwood with regard to *P. ramorum* and *P. kernoviae*–infected *Fagus* and *Quercus* in the U.K.

Movement of *P. ramorum* and *P. kernoviae* by People

At the first SOD meeting in January 2002, Tjosvold, Davidson and colleagues produced some striking data on the presence of *P. ramorum* inoculum on hikers' boots. *Phytophthora ramorum* was sampled from the soil of hikers' shoes after they walked 2.4 km on a trail at the Sonoma Fairfield Osborn reserve. Soil from shoes yielded 46.7 percent and 33.3 percent positive samples during the two trials in April 2003 (Davidson and others 2005). Recently Webber and Rose (these Proceedings) working at sites in Cornwall, U.K. have collected samples from boots of people leaving *P. kernoviae*-infested woodland, mainly within the official *P. kernoviae* 'Management Zone'. Again, significant levels of infestation in soil adhering to footwear were detected.

Movement of inoculum on feet of people, animals and on wheeled vehicles is clearly a major potential pathway for local and even international spread of *P. ramorum and P. kernoviae*. Yet at most *P. ramorum* infested sites in California and Oregon access by people and vehicles for recreational or employment purposes continues as normal. The same is true for *P. ramorum* and *P. kernoviae* infested sites in Cornwall, U.K. Indeed in Cornwall, some of the infested woodland sites are popular pheasant shooting venues in winter, just when the soil may be at its wettest. As a consequence, movement of infested soil is likely between local shooting estates. Some shooting clients may fly in from as far away as the U.S. What is on their boots when they arrive home?

Movement of *P. ramorum* and *P. kernoviae* inoculum on feet of humans or animals and on wheels of machinery, is probably another of those 'inconvenient truths'. If we are serious about management and control of *P. ramorum* and *P. kernoviae* we may have to severely modify our behaviour in terms of public access to affected sites. Maybe we need to learn from the two already historically well documented situations where inoculum of invasive *Phytophthora* species has been shown to spread on feet and machinery: *P. cinnamomi* in jarrah forests and native vegetation in western Australia (for example Brandis and Batini 1985, Colquhoun and Hardy 2000, Shearer and Tippett 1989) and *P. lateralis* in Port-Orford-cedar areas in the Pacific northwest (for example. Hansen and others 1999; Goheen and others 2006). In these situations strict management regimes have been developed locally including seasonal 'no go' areas and stringent vehicle washing protocols. Similar regimes may be needed for *P. ramorum* and *P. kernoviae* and for other invasive *Phytophthora* species in future.

Concluding Thought: How Many Undescribed *Phytophthora* species?

Hawksworth (2001) estimated that 90 percent of fungi were still unknown to science. About 60 described *Phytophthora* species were known in 2001 (today the figure is about 80 described species). Based on the above admittedly loose estimate, it is possible that there are as many as 540 still undescribed *Phytophthora* species in unexplored ecosystems. More conservatively, it seems reasonable to propose both from the above 90 percent estimate and from the many new species currently being described, that the total number of *Phytophthora* species may lie between 200 and 600. Say 10 percent of these undescribed species were potentially invasive or seriously damaging forest *Phytophthora* species (about 5 to 10, or 8 to 16 percent of the 60 known species in 2001 fall into this category, depending upon how one defines a 'potentially invasive or seriously damaging forest *Phytophthora'*). Then there could be some 10 to 50 unknown potentially damaging forest *Phytophthora* species still out there in their natural habitats, waiting to be introduced into a new evolutionary playground by man. Tomorrow's *P. ramorums*, *P. kernoviaes*, *P. alnis*, *P. cinnamomis*. . . . .

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What You Can Do to Help Improve Regulation of the Plants for Planting Pathway[1]

Kerry O. Britton[2]

Abstract

The current rules for plants for planting are being revised. The United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) has already provided several opportunities for public input into the new rules, and more opportunities will be coming soon. Considering the importance most plant health scientists attach to this issue, it is surprising how few people contribute comments. It is as easy as sending an email, once you sign up for notices. The proposed changes to the rules are outlined in this paper, with suggestions for research needs to inform the regulatory process and its effective implementation. It is up to YOU to do something about this important pathway!

Key words: Nursery stock, plants for planting, nursery stock imports, nursery stock regulation, nursery stock trade.

Introduction

The current rules for plants for planting could be described as a "Black List" approach. Under this system, all plants for planting are permitted entry unless they are known to harbor a specific regulated pest. No rating of weediness is currently required of imported plants. At present, 106 genera or species are prohibited based on concerns about specific pest/host/country combinations (See further reading, 1). There are 56 genera or species which require post-entry quarantine. Such quarantine is usually just a period of separation from saleable stock in a corner of a grower's facility. There are no longer any limitations on the number of plants that can be shipped, and routine fumigation is no longer required. Plants can be imported for propagation or for retail sales, as long as they pass through a plant inspection station, where approximately 2 percent are inspected for pests.

Issues

Too many plants to inspect

In 2006, 2.5 billion plants were imported into the U.S. Of these, about 75 percent enter via the Miami Port Inspection Station, where 22 inspectors struggle valiantly to keep up with the flow.

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Latent pathogens and hidden insects

All pathogens and many insect pests have latent periods before symptoms are expressed. By definition these cannot be detected by visual inspection.

Most serious forest pathogens and pests have been unknowns

Many pests are unknown to science prior to establishing invasive populations in a new land. Expecting overloaded inspectors to detect unknown pathogens is unrealistic.

Too many risk assessments to do

The tide of import plants is overwhelming, and to do a risk assessment on each plant/country of origin combination would take several lifetimes. Therefore in most cases the risk associated with plants we are importing has not been assessed.

Legal Limitations

The Sanitary and Phytosanitary Agreement of the World Trade Organization specifies that countries cannot require phytosanitary measures against non-regulated pests. To be regulated, a pest must not be present, or it must be limited in distribution AND under regulatory control.

An International Standard is Needed

The legal limitations listed above prevent regional or national plant protection organizations from taking a strong stand against pests arriving on plants for planting. This problem must be fixed at the International Plant Protection Convention (IPPC) level, by developing an international standard. The IPPC can ensure a level playing field and avoid claims of trade protectionism. The IPPC recently convened a committee to draft an international standard on plants for planting, but it undoubtedly will take years to gain the unanimous approval required for adoption.

The NAPPO Standard

The North American Plant Protection Organization (NAPPO) recently adopted RSPM No. 24, a regional standard for plants for planting (see further reading, 2). This standard calls for a systems approach for producing clean nursery stock, which will be bilaterally negotiated crop by crop, country by country. The presumption is that preventing pests we know will reduce the incidence of unknowns too. Each member country (in other words, Canada, Mexico, and the U.S.) has agreed to develop regulations to implement the standard. The responsibility for this lies with each national plant protection organization (in the U.S. this is the USDA_APHIS).

The USDA Regulations for Plants for Planting

APHIS is currently revising these regulations, called “Q-37”. This process was begun years before the NAPPO standard was adopted. Adjustments will have to be made to help bring USDA regulations in line with the NAPPO standard. This is very much a work in progress, so plant health specialists should not hesitate to contribute their expertise toward the goal of making trade in plants for planting as safe as it can

be. To sign up for notices of opportunities to comment, go to: https://web01.aphis.usda.gov/PPQStakeWeb2.nsf.

An advanced notice of proposed rulemaking was issued in the federal register in 2004 (see further reading, 3). It solicited feedback on the following proposals, which seek to address plants as pests themselves, as well as plants as pathways for pests:

1) Collect consistent entry data

Currently the certificates accompanying plants often do not provide the scientific name or even the genus of plants being imported, and quantity estimates are unreliable. APHIS is already developing better tracking systems.

2) Create a new category: Not authorized for import pending a pest risk assessment (NAPPRA)

This category would be populated by plants which meet the following criteria:

- Damage potential demonstrated
- Pest clearly identified
- Geographic regulatory requirements (in other words., not here, or not widespread)
- Host range of pest defined (at either the genus or species level)
- Status of host as pathway clearly documented

3) Establish clean stock programs and best management practices

The nursery industry has been working towards a voluntary program within the U.S., but the implementation of RSPM-24 will require certification of growers by the national plant protection organization of countries exporting plants to North America.

4) Streamline the pest risk assessment (PRA) process

Some PRAs take years to complete, and the flow of desired trade plant x origin combinations is much greater than the capacity of APHIS to assess their risks.

5) Consolidate the plants for planting regulations

A large number of rules for different hosts and noxious weeds have been recorded over the years.

In the years since this advance notice was published, APHIS has wrestled with the feedback they received, and also with the necessity of implementing the NAPPO standard. Early in 2007 they hosted an online forum on assessing weediness of plants proposed for importation into the United States.

Plant Health Specialists Voice Concern

A new and very readable report by The Nature Conservancy provides a good description of the situation (see further reading, 4). The writers propose that all plants be put in the NAPPRA category, and that APHIS move quickly to clear most common imports, keeping natural resources safe while deciding whether the others require restrictions. There is considerable support for a stringent approach like this in the international community of plant pathologists.

A concept paper on plants for planting was recently developed by the International Union of Forest Research Organization's working group on invasive species in international trade (see further reading, 5). The paper calls for a pathway approach to plants for planting, using the new wood packing materials regulations as an example. However, it is important to recognize that the pathway risk assessment as described by the IPPC requires the identification of specific exemplary pests and the adoption of mitigation measures that address those pests. Again, the presumption is that by mitigating pests we know, we will also serendipitously mitigate many unknown pests.

What can we ALL do to Help?

Stay informed (see further reading, 6) and engage in the discussion! Spread the word: plants for planting is a key pathway. Encourage your local nurseries to sell certified stock, and be willing to pay a little extra for it. Keep your eyes open and help identify new invasive plants and pests.

What can Scientists do?

The following areas of information are needed for most invasive species:

- Criteria for invasiveness: what makes a species invasive?
- Clean stock production systems that are practical and effective.
- Taxonomy, distribution, and host range of pest organisms.
- Detection methods for use at ports, in preclearance programs, and in nurseries.
- Estimates of impacts (actual and potential) to guide decisionmakers.
- Mitigation measures that will permit trade to continue, safely.

What can the Nursery Industry do?

- Help frame best management practices that are practical, effective, and proven.
- Help consumers appreciate native and home-grown products.

Summary

Recognizing that we have a problem is the first step toward finding a solution. The system we have now allows those who import plants for fun or profit to do so with little consideration for the potential long-term impacts on natural ecosystems. The government must then combat the hitchhiking pests or weeds using millions of tax dollars. A system should be devised that places the economic burden of screening plants for planting on those who desire the plants. I would gladly pay a little extra for my exotic plants to be secure in the knowledge that my pleasures are not causing ecological harm, or jeopardizing the inheritance of future generations.

Acknowledgments

Thanks to all the hard working, dedicated people at APHIS who are truly stuck with a difficult job, trying to enhance trade while protecting natural resources.

Further Reading

1. For the list of prohibited plants, and those allowed with restrictions, see: http://ecfr.gpoaccess.gov/cgi/t/text/textidx?c=ecfr&sid=64938ae67d906f1fc8ba68b478241a07&rgn=div8&view=text&node=7:5.1.1.1.6.7.49.8&idno=7

2. RSPM No. 24 Integrated Pest Risk Management Measures for the Importation of Plants for Planting into NAPPO Member countries. Adopted October, 2005 http://www.nappo.org/Standards/NEW/RSPMNo.24-e.pdf

3. U.S. Department of Agriculture Animal and Plant Health Inspection Service Advance notice of proposed rulemaking and request for comments on nursery stock regulations Federal Register: December 10, 2004 (Volume 69, Number 237) http://a257.g.akamaitech.net/7/257/2422/06jun20041800/edocket.access.gpo.gov/2004/pdf/04-27139.pdf

4. An Ounce of Prevention: How to Stop Invasive Insects and Disease from Devastating U.S. Forests http://www.nature.org/initiatives/forests/files/ounceofpreventionsingle1.pdf

5. Recommendation of a Pathway Approach for Regulation of Plants for Planting. A Concept Paper from the IUFRO Unit on Alien Invasive Species and International Trade http://www.forestry-quarantine.org/Documents/IUFRO-ConceptPaper-%20Plants-Planting.pdf

6. For more information on the developing Plants for Planting regulations, see: http://www.aphis.usda.gov/ppq/Q37/revision.html

Review of Current Information Regarding the Phytosanitary Risks of *Phytophthora ramorum* and North American Conifers[1]

Brenda Callan,[2] Shane Sela,[3] and Eric Allen[2]

On March 3, 2007 the North American Plant Protection Organization (NAPPO) sponsored a "Risks to Conifers" discussion panel to review the state of scientific knowledge regarding *Phytophthora ramorum* Werres, De Cock & Man in 't Veld and conifers and the potential for the pathogen to be transported with conifer forest products moving in international trade. This panel took place during the third Sudden Oak Death Science Symposium organized by the USDA Forest Service Pacific Southwest Research Station, and the California Oak Mortality Task Force (COMTF) in Santa Rosa, California. It was attended by over 100 participants, including researchers and forest managers from the United Kingdom, the Netherlands, Belgium, the United States, and Canada. A number of reviews were presented by leading scientists in the field of *P. ramorum*, followed by a general discussion on the gaps in scientific knowledge. A list of presenters and the titles of their talks is available at: http://nature.berkeley.edu/comtf/sodsymposium/schedule_sections.htm#risktoconifers

Current information indicates that only a few conifers are proven natural hosts of *P. ramorum* including: *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir), *Sequoia sempervirens* (Lamb.) Endl. (coastal redwood) and *Taxus baccata* L. (common yew, not native to North America) (Davidson and others 2002, Maloney and others 2002, Lane and others 2004). Other North American conifer species have been observed in nature to demonstrate symptoms of the disease but have not been conclusively proven as hosts. In particular, *Abies grandis* (Douglas ex D. Don) Lindley (grand fir), *A. concolor* (Gord. & Glend.) Lindl. ex Hildebr. (white fir) and *Taxus brevifolia* Nutt. (Pacific yew) are listed as associated hosts (COMTF). Most of these plants are present in the most heavily affected areas of northern California where inoculum levels are extreme. Scientists currently agree that high inoculum levels appear to be essential for infection to occur in these species under natural conditions. Chastagner (2005) reported that in laboratory inoculation trials, more than 10,000 spores/ml were necessary to cause infection in Douglas-fir and grand fir.

Hansen (2007) reported that in Oregon where the disease is present primarily in a mixed evergreen - *Lithocarpus densiflorus* (Hook. & Arn.) Rehd (tanoak) woodland, *P. ramorum* infection has been observed in only one instance on Douglas-fir saplings located directly beneath an infected tanoak. On private forest lands in Oregon, Douglas-fir and coastal redwood seedlings have been routinely planted in areas where eradication treatments have occurred and have also been planted as "bait" seedlings adjacent to known infested tanoak stumps. These and other planted seedlings have been followed since 2003. *Phytophthora ramorum* has not been recovered from these planted seedlings or nearby hosts in annual monitoring to date, despite the persistence of inoculum in the soil (Goheen this Proceedings).

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Hansen (2007) also noted that in observations of conifers in Europe, infection has not been observed, even though the plants are adjacent to heavily infected rhododendrons.

Chastagner (2007) reported that in a Christmas tree plantation in California, symptom expression was limited to the plants within about 5 m of heavily infected *Umbellularia californica* (Hook. & Arn.) Nutt (California bay laurel) plants surrounding the plantation. Chastagner (2007) also reported that no sporulation has been detected on naturally infected Douglas-fir and grand fir shoots, which suggests that there is a low risk of spread from these conifers. Infection of Douglas-firs and grand firs in the plantation was restricted to emerging shoots that resulted in flagging at branch tips or canker development on branches, but stem infections were never observed. Until the recent recovery of *P. ramorum* from Pacific yew stems in the wild (Bienapfl and others 2006), the occurrence of the pathogen in wood of naturally infected conifers has never been reported, although lab inoculations demonstrate that it is possible.

Coastal redwood was reported by Garbelotto (2007) to be the third most commonly infected plant in the *P. ramorum* infection zone in northern California. However, infection levels of coastal redwood are relatively small when compared to the levels of infection in tan oak and bay laurel.

Numerous other conifers have been artificially tested. Webber (2007) reported that more than 40 species had been tested in the United Kingdom (U.K.) and many of these artificial inoculations have been reported on the U. K.'s Central Science Laboratories' website: Risk Analysis for *Phytophthora ramorum* (http://rapra.csl.gov.uk). Hansen (2005), Chastagner and others (2005) and others have undertaken similar laboratory inoculations. Artificial inoculations of *Pinus*, *Picea*, *Larix* and other conifers have shown varying degrees of host response. Most show similar symptoms to those susceptible conifers found in nature including tip-dieback and shoot blight. Some of have also shown lesions in the wood when the pathogen is inoculated into the stem. Garbelotto (2003) reported lesions in the stems of *Pinus radiata* D. Don and Brasier and others (2005) reported lesions occurring in *Picea* following stem inoculation. However, most scientists conclude that laboratory inoculations are difficult to extrapolate to forest conditions, given that spore loads necessary to cause infection may not occur in natural environments.

In conclusion, panel participants agreed the risks of conifer commodities (lumber, logs, and os forth) carrying the pathogen to new areas is extremely low. To date, the known incidence of long distance pathogen movement has been restricted to movements of infected nursery stock, particularly high risk hosts such as rhododendron, the movement of infected soils or the movement of more highly susceptible deciduous plant parts such as leaves and branches. It is possible that conifer plant parts such as branches may pose a risk for moving the pathogen, but transmission of the pathogen by this method or even sporulation on conifer branches has not been observed. However, potential for sporulation of the pathogen on conifer foliage and green succulent tissues should be investigated. A number of conflicting reports on the thermal requirements to kill *P. ramorum* in wood (particularly in deciduous wood) demands further analysis. Finally, although the likelihood of the pathogen moving within the wood of conifers appears very low, the potential that conifer logs felled within infested stands (in other words in bay laurel, rhododendron, or tanoak woodlands) may carry contaminating spores should also be investigated. It is unclear, if in such circumstances sufficient spores could survive transportation and be distributed to cause infection elsewhere.

Key words: *Phytophthora ramorum*, sudden oak death, conifers, phytosanitary risks.

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Biology and Ecology

Umbellularia californica

Phytophthora ramorum Infects Tanoak Sapwood and is Associated With Reduced Sap Flux and Specific Conductivity of Xylem[1] [2]

Jennifer Parke,[3] Eunsung Oh,[4] Steve Voelker,[5] Everett Hansen,[6] Gerri Buckles,[7] and Barb Lachenbruch[8]

Abstract

Culture, detection with diagnostic PCR, and microscopy demonstrated the presence of *Phytophthora ramorum* in the sapwood of mature, naturally infected tanoak *(Lithocarpus densiflorus)* trees in Curry County, Oregon. The pathogen was strongly associated with discolored sapwood ($P<0.001$), and was recovered or detected from 83 percent of discolored sapwood tissue samples. Hyphae were abundant in the xylem vessels, ray parenchyma, and fiber tracheids. Chlamydospores were observed in the vessels**.** A field study was conducted to determine if trees with infected xylem had reduced sap flux and reduced specific conductivity relative to non-infected control trees. Sap flux was monitored with heat-diffusion sensors, and tissue samples near the sensors were subsequently tested for the presence of *P. ramorum.* Adjacent wood sections were excised and specific conductivity was measured in the laboratory. Both sap flux and specific conductivity were significantly reduced in infected trees as compared to non-infected control trees. Vessel diameter distributions did not differ significantly between the two treatments, but tyloses were more abundant in infected than in non-infected trees. Reduced sap flux and specific conductivity may result from increased embolism caused by *P. ramorum* infection, the presence of fungal structures, and the increased abundance of tyloses present in the vessels. Reduced stem water transport may contribute to crown mortality associated with sudden oak death.

Key words: *Lithocarpus densiflorus*, water relations, pathogenesis, embolism, tyloses.

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Invasion of Xylem of Mature Tree Stems by *Phytophthora ramorum* and *P. kernoviae*[1]

Anna Brown[2] and Clive Brasier[2]

Abstract

The aetiology and frequency of *Phytophthoras* in discoloured xylem tissue beneath phloem lesions was investigated in a range of broadleaved trees infected with *P. ramorum, P. kernoviae* and several other *Phytophthoras*. Isolation was attempted from the inner surface of 81, 6 x 4 cm sterilised discoloured wood panels from 53 trees. Discolouration mostly extended 1 to 5 mm into the xylem (75 percent) but incursions of 6 to 10 mm (10 percent) and 10 to 25 mm (15 percent) were frequent. *Phytophthora* was isolated from 81 percent of the wood panels. In 66 cases, both a wood panel and an overlying phloem panel were sampled. In 56 percent of these *Phytophthora* was isolated from both the wood and the phloem panel. In 23 percent it was isolated from the wood panel only and in 8 percent from the phloem panel only.

Small 'island' phloem lesions, often in linear arrays adjacent to main lesions, were a common feature of *Fagus* and *Quercus* trees infected with *Phytophthora ramorum* or *P. kernoviae*. Island lesions were often connected by underlying strips or intermittent pits of discoloured xylem in line with the wood grain. *P. ramorum, P. kernoviae* and other *Phytophthoras* were successfully isolated from these connecting xylem features. *P. ramorum* and *P. kernoviae* were also recovered from discoloured tissue 5 to 25 mm below exposed xylem surfaces 12 to 24 months after the overlying phloem was removed.

These results show that *Phytophthoras* commonly occupy xylem beneath phloem lesions; that they can perennate in xylem tissue; that they can spread in xylem tissue ahead of phloem lesions; and indicate that they may initiate new phloem lesions in this way. Such colonisation must lead to at least local xylem dysfunction. It is recommended that, if xylem discolouration is present, isolation of *Phytophthora* should be attempted from the xylem as well as the bark. Also, that removal of infected outer sapwood should be undertaken during excision of *Phytophthora* bleeding lesions for disease control and in protocols aimed at preventing national or international spread of tree stem *Phytophthoras*.

The results from this work are covered in more detail in Brown, A.V.; Brasier, C.M. 2007. Colonization of tree xylem by *Phytophthora ramorum* and *P. kernoviae* and other *Phytophthora* species. Plant Pathology. 56: 227–241.

Key words: *Phytophthora*, bark lesions, bleeding cankers, phloem, tree stem diseases, wood, xylem.

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Chemistry of Coast Live Oak Response to *Phytophthora ramorum* Infection [1]

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Abstract

Since the mid 1990s, *Phytophthora ramorum* has been responsible for the widespread mortality of tanoaks, as well as several oak species throughout California and Oregon forests. However, not all trees die, even in areas with high disease pressure, suggesting that some trees may be resistant to the pathogen. The apparent resistance to *P. ramorum* infection of some individuals within coast live oak populations has been observed in artificial inoculation studies. For example, from artificial branch-cutting inoculation trials, Dodd and others (2005) found significant variation (up to eightfold difference in lesion sizes) in susceptibility to *P. ramorum*. In addition, apparent resistance has also been observed in naturally infected forests, where a number of coast live oaks have survived for more than seven years despite being infected (McPherson and others 2005 and unpublished data).

Elevated levels of secondary metabolites, specifically phenolic compounds in infected tissue, are often associated with resistance to fungal pathogens in angiosperms (Bennett and Wallsgrove 1994; Ostrofsky and others 1984). It is possible that these apparently resistant coast live oaks may have increased amounts of phenolic compounds in the *P. ramorum* infected tissue, which is inhibiting the growth of the pathogen. However, there are no reports that describe the changes in secondary metabolites of coast live oaks infected with *P. ramorum*. To date, the majority of studies investigating phenolic chemistry in oak have focused on constitutive wood and foliage chemistry.

Three field experiments were carried out in Deer Island and China Camp State Park, CA between December 2004 and September 2005 on large trees (DBH approx. 28 to 69 cm). Trees were either artificially inoculated (experiments 1 and 3) or naturally infected with *P. ramorum* (experiment 2). Phloem was sampled from the margin of active lesions and also from healthy phloem at least 60 cm away from lesion margin (AFC, away from cankers) of some of the same inoculated trees and from apparently healthy trees. Phenolics were extracted in methanol, identified by HPLC-mass spectrometry or matched to standards and quantified by HPLC-UV analysis. Nine phenolic compounds (gallic acid, catechin, tyrosol, a tyrosol derivative, ellagic acid and four ellagic acid derivatives) were analyzed in this way.

Significant differences in phenolic profiles were found between phloem sampled from the active margins of cankers, healthy phloem from asymptomatic trees, or AFC phloem,

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although the magnitude and direction of the responses was not consistent across all experiments. Concentrations of gallic acid, tyrosol, and ellagic acid showed the greatest differences in these different tissues, but varied considerably across treatments. Specifically, significantly greater amounts of ellagic acid and gallic acid were observed in infected phloem than non-infected phloem in experiments 1 and 2. In contrast, significantly higher amounts of tyrosol and ellagic acid were present in infected phloem than in corresponding controls in experiment 3 (tables 1-3). Interestingly, catechin levels were significantly reduced in infected tissue in two of the three experiments, i.e. experiments 1 and 3.

Table 1—Effect of artificial *P. ramorum* inoculation on the concentration of nine phenolic compounds extracted from the phloem of coast live oak in experiment 1

	Experimental factors*	
Compound	Infected (N = 5)	Healthy (N = 5)
Gallic Acid	1.97 (0.93) a	0.09 (0.03) b
Tyrosol	0.91 (0.35)	1.01 (0.18)
TY1[x]	2.88 (0.77)	4.08 (0.69)
Catechin	0.63 (0.16) a	2.87 (0.35) b
EA1[y]	0.11 (0.05)	0.39 (0.16)
EA2[y]	0.03 (0.01) a	0.37 (0.17) b
Ellagic acid	1.47 (0.69) a	0.02 (0.01) b
EA3[y]	0.11 (0.06	0.35 (0.09)
EA4[y]	0.09 (0.06)	0.10 (0.02)

*All concentrations expressed as mg/g fresh weight (SE).
[x] Compound quantified in terms of tyrosol equivalents.
[y] Compounds quantified in terms of ellagic acid equivalents.
Values in each row followed by different letters are significantly different ($P < 0.05$).

Table 2—Effect of natural *P. ramorum* infection on the concentration of nine phenolic compounds extracted from the phloem of coast live oak in experiment 2

	Experimental factors*		
Compound	Infected (N = 7)	AFC (N = 7)	Healthy (N = 5)
Gallic Acid	0.94 (0.29) a	0.09 (0.02) b	0.07 (0.01) b
Tyrosol	0.82 (0.25)	1.13 (0.20)	1.26 (0.17)
TY1[x]	2.89 (0.56)	2.71 (0.34)	2.58 (0.23)
Catechin	3.52 (0.59)	3.32 (0.54)	2.07 (0.19)
EA1[y]	0.36 (0.13)	0.43 (0.15)	0.32 (0.06)
EA2[y]	0.15 (0.05)	0.31 (0.16)	0.14 (0.05)
Ellagic acid	0.16 (0.05) a	0.04 (0.02) b	0.04 (0.02) ab
EA3[y]	0.21 (0.10)	0.20 (0.12)	0.10 (0.10)
EA4[y]	0.13 (0.06)	0.15 (0.07)	0.09 (0.08)

*All concentrations expressed as mg/g fresh weight (SE).
[x] Compound quantified in terms of tyrosol equivalents.
[y] Compounds quantified in terms of ellagic acid equivalents.
Values in each row followed by different letters are significantly different ($P < 0.05$).

Table 3—Effect of artificial *P. ramorum* inoculation on the concentration of nine phenolic compounds extracted from the phloem of coast live oak in experiment 3

	Experimental factors*	
Compound	**Infected**	**AFC**
Gallic Acid	0.42 (0.30)	0.47 (0.21)
Tyrosol	2.23 (0.33) a	0.75 (0.57) b
TY1[x]	2.19 (0.87)	4.25 (1.32)
Catechin	1.18 (0.34) a	3.84 (0.88) b
EA1[y]	0.21 (0.13)	0.40 (0.16)
EA2[y]	0.18 (0.12)	0.37 (0.18)
Ellagic acid	0.53 (0.16) a	0.04 (0.01) b
EA3[y]	0.07 (0.03)	0.23 (0.10)
EA4[y]	0.14 (0.06)	0.12 (0.05)

*All concentrations expressed as mg/g fresh weight (SE).
[x] Compound quantified in terms of tyrosol equivalents.
[y] Compounds quantified in terms of ellagic acid equivalents.
Values in each row followed by different letters are significantly different ($P < 0.05$).

The soluble phenolic compounds identified in the phloem extracts of infected coast live oak have been implicated as playing key roles in defense against fungi and herbivores in many woody species, including other *Quercus* spp. (Feucht and Treutter 1999; Malterud and others 1985, Pearce 1996). For example, the durability of some hardwood species against microbes has been directly attributed to the elevated presence of hydrolysable tannins (Barry and others 2001, Hillis 1999, Klumpers and others 1994, Vivas and others 2004).

The antifungal activities of gallic acid (2, 3, 6 and 10 mg/mL) and tyrosol (1, 2, 3 and 6 mg/mL) were tested against *P. ramorum, P. cinnamomi, P. citricola,* and *P. citrophthora in vitro*. Both compounds showed strong dose-dependent inhibitory effects against all four species (fig. 1).

In conclusion, this study demonstrated clear host secondary metabolite responses that may be implicated in resistance of coast live oak to attack by *P. ramorum*. Further studies involving correlation of compound concentrations with disease resistance *in planta* will be necessary to establish a potential defensive role for any of these compounds. If such a role is established, then some of these compounds could be used as biomarkers in the selection of resistant coast live oak genotypes. These studies, however, are contingent on developing reproducible techniques that can be used routinely to obtain quantitative measures of host resistance (e.g. Blodgett and others. 2007), which are lacking at present.

Key words: *Phytophthora ramorum,* sudden oak death, defense, resistance, phenolics.

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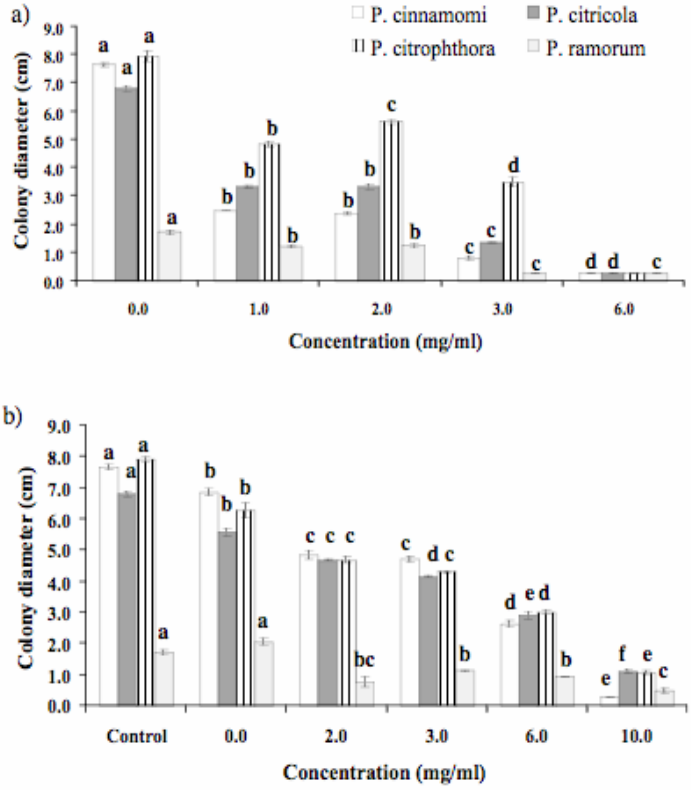

Figure 1. Effects of tyrosol (a) and gallic acid (b) on colony growth rates for *P. cinnamomi, P. citricola, P. citrophthora,* and *P. ramorum*. Each bar represents the mean of three replicates ± 1 SEM and includes the diameter of the original inoculum plug (3 mm). Different letters indicate significant differences ($P < 0.05$, LSD test) within species only. Species was a significant factor in all ANOVAs, indicating that the four *Phytophthora* species are affected differentially by both treatments. The control for tyrosol (0.0 mg/ml) was used as a baseline and is reproduced as the control bars in chart B.

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Log Susceptibility of Iberian Tree Species to *Phytophthora ramorum*[1]

Eduardo Moralejo,[2] José Andrés García-Muñoz,[2] and Enrique Descals[2]

Abstract

Phytophthora ramorum is a plant pathogen introduced into Europe and North America. It can infect any host species belonging to different botanical families within the seed plants. Such infective capacity indicates that it can overcome basic plant defence responses that have been phylogenetically conserved in plants (Heath 1991). In addition, *P. ramorum* is capable of infecting woody plants that are not predisposed (i.e. physiologically healthy) and can colonize a diversity of ecological niches such as leaves, stems, trunks and maybe roots and fruits. All these traits make *P. ramorum* potentially invasive to many ecosystems worldwide. Despite this, what renders a plant species susceptible to *P. ramorum* is not well understood, but it is becoming evident that trees of certain plant families, such as the Fagaceae (*Fagus*, *Castanopsis*, *Castanea*, *Quercus*, and so forth), are much more predisposed to developing trunk cankers caused by this pathogen than any other tree species.

As part of the Risk Analysis for *Phytophthora ramorum* (RAPRA) project, a long-term study on the risk posed by *P. ramorum* to European natural ecosystems, the capacity of the pathogen to colonize the inner bark of some Iberian tree species was assessed. Twenty-one tree species were selected, including hygrophilous conifers, evergreen drought-resistant species, and those covering more than 80 percent of the overall forest surface of the Mediterranean and sub-Mediterranean vegetation of the Iberian Peninsula (Spain and Portugal). We used the log inoculation method described by Brasier and Kirk (2001) which has been used to assess the susceptibility of trees to *Phytophthora* species in the UK. Logs were cut from eight different individual trees per species in summer (June toJuly/2005 to 2006) and winter (Dec. to Jan. 2004 to 2006), and inoculated a day after returning to the laboratory. Five isolates, three belonging to the European lineage (EU1) and two to the North American lineage (NA1), were used. One isolate of *P. cinnamomi*, a well-known pathogen of oaks, and *P. hedraiandra*, a recently described species, were included as positive controls. A single sterile carrot agar plug was used to inoculate each log and served as the negative control. Eight logs (10-20 cm diameter x 1.2 m long) per tree species were wound-inoculated with 7 mm diameter mycelial plugs at eight equidistant points (Brasier and Kirk 2001). At each of the eight inoculation sites, a different isolate, species, or control was used. The trunks were sealed with double polyethylene film to ensure suitable humidity and incubated at 20°C in a quarantine chamber. After approximately 40 days, the outer bark was removed, the outline of the necrotic lesion traced on transparent paper and the image scanned. The lesion area was calculated with the Olympus DP12 Soft version 3.2.

Most of the *Quercus* species ranked in the highly susceptible category with mean lesion areas on *Q. pyrenaica*, *Q. humilis*, *Q. canariensis*, *Q. faginea*, and *Q. suber* over 100 cm^2 and as

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high as 400 cm^2 in trials carried out in summer; *Q. ilex* ranked in the moderately susceptible category forming lesions up to 130 cm^2. As expected, *P. cinnamomi* was as highly aggressive to *Quercus* spp. as *P. ramorum*, but *P. hedraiandra* was considerably less aggressive. However, occasionally *P. hedraiandra* also caused large lesions (> 100 cm^2) on *Quercus* spp. The holm oak (*Q. ilex*), cork oak (*Q. suber*) and Portuguese oak (*Q. faginea*) were on average slightly susceptible in the winter trials, although some individuals attained large lesion areas suggesting that some kind of a threshold effect occurred in the interaction between host-pathogen genotype pairs (*cf.* summer trials). Cork oak trees exhibited rounded bleeding lesions similar to those observed on *Q. agrifolia* in California, and the other *Quercus* species typically formed diamond-shaped lesions. Of the pines inoculated, only *Pinus halepensis* formed considerably long narrow lesions. The other four pines, *P. pinaster*, *P. pinea*, *P. nigra* and *P. sylvestris*, were resistant. Two trees belonging to the Oleaceae, *Fraxinus angustifolia* and *Olea europea*, were consistently resistant to all the *Phytophthoras* inoculated. On average, the three European isolates of *P. ramorum* formed larger lesions on susceptible hosts than the two American isolates, although differences were not statistically significant. There was also evidence of host variation in the susceptibility within a species and across seasons among some tree species tested. Our results fit the general pattern of oak susceptibility and pine resistance, and of seasonality and individual (within population) variation observed by other researchers on log inoculations performed on trees of northern Europe and North America (Brasier and others 2006, Hansen and others 2005).

Our study indicates that the living inner bark of *Quercus* species collected in the Iberian Peninsula is very susceptible to *P. ramorum*. However, we have not yet tested whether the outer bark can be an important barrier to infection by zoospores under natural conditions. At least four species (*Q. ilex*, *Q. suber*, *Q. humilis* and *Q. canariensis*) thrive in extensive areas of Spain and Portugal with climatic conditions comparable to those where sudden oak death occurs in California. Since the foliage of an important number of understorey plants accompanying these trees has been shown to be susceptible *in vitro* to *P. ramorum* (Moralejo and others 2006a), and because this foliage is capable of sustaining sporulation (Moralejo and others 2006b), we consider that there is a high risk of the pathogen becoming established and provoking tree mortality in the Iberian Peninsula. At the local scale, our results highlight the risk posed especially to forest ecosystems in southern Spain (e.g. the Alcornocales Natural Park) where relict populations of *Q. canariensis* grow with *Rhododendron ponticum*, *Viburnum tinus* and other potential host species of the understorey.

Key words: Invasive alien species, oak woodlands, *Phytophthora hedraiandra*, plant pathogen, trunk canker.

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Infection of Tree Stems by Zoospores of *Phytophthora ramorum* and *P. kernoviae*[1]

Clive Brasier[2] and Anna Brown[2]

Abstract

The invasive *Phytophthora ramorum*, *P. kernoviae,* and other aerial *Phytophthoras* are causing bleeding lesions on the trunks of mature trees, especially beech (*Fagus sylvatica*), in Cornwall, southwest England. The relationship between the results of host susceptibility tests using wound inoculation of excised logs in the laboratory and field observations in Cornwall has generally been good. However there have been some discrepancies. For example European sycamore (*Acer pseudoplatanus*) is moderately to highly susceptible to *Phytophthora ramorum* and *P. kernoviae* in the lab tests but is rarely susceptible in the field, although frequently exposed to natural inoculum. This suggests the possibility that host resistance is operating at two levels: at the bark surface-resistance to initial zoospore penetration; and in the phloem once the pathogen has gained entry. Little is known about the ability of zoospore inoculum to directly penetrate intact tree bark, although this is the presumed mode of entry of *Phytophthoras* above ground level. We are investigating this issue by examining the ability of zoospores to penetrate unwounded stems using both laboratory generated and natural zoospore inoculum. We summarize here research in progress.

In a preliminary laboratory test, *P. ramorum* zoospore suspensions were placed in plastic tubes attached to unwounded surfaces of freshly cut 50 cm long x 12 cm diameter stems of several tree species using modelling clay. After two weeks the inoculum source and outer bark were removed. Lesions of up to 5 cm were observed in the phloem of *F. sylvatica*, *Castanea sativa*, *Quercus rubra,* and *Picea sitchensis* and the pathogen was re-isolated, confirming zoospore penetration of unwounded bark. Lesions were absent in *Q. robur* but the pathogen could be re-isolated from visually healthy phloem beneath the inoculum points, in other words the bark was again penetrated although no lesions developed.

In a similar experiment, zoospores of *P. kernoviae*, *P. citricola,* and *P. cambivora* also caused phloem lesions in logs of *F. sylvatica.* Data from a large experiment, involving logs of *F. sylvatica, Q. robur,* and *A. pseudoplatanus* and laboratory produced zoospores of *P. ramorum*, *P. kernoviae*, *P. citricola,* and *P. cambivora*, could not be utilised owing to an adverse host response to the commercial adhesive used for attaching the plastic tubes. It is hoped to repeat the experiment in future with modelling clay or another benign adhesive.

To examine the comparative potential for infection in the field, freshly cut sealed logs 60 cm long x 15cm diameter of *F. sylvatica, Q. robur,* and *A. pseudoplatanus* were placed under a canopy of 2 to 4 m tall rhododendrons heavy infected with either *P. ramorum* or *P. kernoviae* at two woodland sites. Potential inoculum was assumed to be of zoospore origin coming from

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splash dispersed sporangia. Twelve logs of each species were used at each site, half being examined for lesion development after 6 weeks and half after 11 weeks.

The experiment was carried out twice. In the first experiment, established in early July 2006, hot dry conditions prevailed from July to mid August and no lesions were found. In the second, established in early August, rain occurred from late August onwards. Lesions caused by both *Phytophthora* species were found on the upper surfaces of the *F. sylvatica* logs only after 6 weeks. By 11 weeks the *P. kernoviae* exposed *F. sylvatica* logs averaged 2.7 lesions per log of mean lesion area about. 23 cm^2; and the *P. ramorum* logs 6.2 lesions per log of mean lesion area only about 8 cm^2. Lesions were therefore more numerous with *P. ramorum* but larger with *P. kernoviae*. The *Phytophthora* species were confirmed by isolation. External bleeds developed in association with a number of the lesions. For both *Phytopthoras*, 36 to 55 percent of the lesions were associated with cut ends of the logs; 33 percent showed no obvious association with a stem feature; and the remainder were associated with fully healed branch stub positions or with live epicormic shoots.

Four lesions (but no bleeds) developed on the six *Q. robur* logs exposed to *P. ramorum* and the pathogen was reisolated from the lesions. No lesions occurred on the *Q. robur* logs exposed to *P. kernoviae*. No lesions developed on any of the logs of *A. pseudoplatanus*. These results are broadly consistent with the relative susceptibility of standing trees of the same host species at these field sites, although this is the first evidence of 'natural' infection of *Q. robur* by *P. ramorum*. It is therefore possible that some *Q. robur* trees become infected by *P. ramorum* in the field without exhibiting external bleeding.

The above data refer to the upper surfaces of the logs only. Destructive sampling revealed that similar numbers of lesions also developed on the bottom surfaces of the *F. sylvatica* logs, probably because the logs were in contact with the litter layer. However the lesions on the bottoms of the logs were much larger than those on the tops, averaging 119 cm^2 for the *P. kernoviae* exposed logs and 62 cm^2 for *P. ramorum*. This difference could reflect the different microclimate at the underside of the logs, such as greater surface moisture levels and a higher phloem water content. Further tests using logs exposed to natural inoculum are planned at these sites, including tests with logs placed on plastic sheeting to prevent contact with inoculum from the litter layer.

Key words: *Phytophthora ramorum*, *Phytophthora kernoviae*, zoospores, bark penetration, phloem lesions.

Phytophthora ramorum Isolated From California Bay Laurel Inflorescences and Mistletoe: Possible Implications Relating to Disease Spread[1]

Gary A. Chastagner,[2] Kathy Riley,[2] and Norm Dart[2]

Abstract

Since 2005, we have been studying the spread and development of *Phytophthora ramorum* at a Christmas tree farm near Los Gatos, California. This research has shown that distance from infected plants, predominantly California bay laurel (*Umbellularia californica*) (referred to as 'bay' throughout), is an important factor relating to the infection of Douglas-fir (*Pseudotsuga menziesii*) and grand fir (*Abies grandis*) Christmas trees at our research site. This abstract reports two case studies involving the possible role that bay inflorescences and mistletoe may play in the spread of *P. ramorum.*

Bay Flowers: In a few instances, we have observed the development of *P. ramorum* induced pitchy cankers on 4 to 5-year-old grand fir branches. These cankers eventually girdle the branch, resulting in branch flagging. To understand the origin of these cankers, we have been trying to determine if the pathogen is: (1) spreading down the branch from infected shoot tips; (2) spreading from infected small interior secondary shoots near the canker, or (3) if there are some conditions that allow for direct infection of the older needles or the bark. Results so far show that: (1) Trees that develop these branch cankers tend to have high levels of shoot infection and it does not appear that the pathogen is spreading asymptomatically down the branches from infected shoot tips. (2) Extensive shoot infections often lead to the development of small, weak epicormic shoots on older branches. Infection of these shoots can result in the development of pitchy cankers. However, in some instances, cankers do not appear to be associated with infected epicormic shoots. (3) Direct infection of older needles and bark might be associated with infected plant debris deposited on the branches of the grand fir trees. Observations led us to consider that detached, dried bay inflorescences, which can be found lodged between needles on grand fir branches, might be a source of inoculum. The work described below details initial investigation of this possibility.

In a few instances we observed that green needles were being shed from the branch surface below a dry bay flower deposited on the branch of grand fir after abscission from the bay tree. Needle shedding is a typical symptom associated with the spread of *P. ramorum* within infected conifer shoots such as Douglas-fir (Hansen and others 2005; Denman and others 2005). In grand fir when the pathogen spreads into the previous year's growth following infection of elongating shoots after bud break in the spring, it is often not visible at first. However, as it spreads down the shoot, the previous year's green needles are shed in the wake of its progression. It is often possible to find the advancing edge of the colonized tissue for isolation by rubbing off the loose needles and then isolating from the area closest to the intact

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needles. To determine if bay flowers might be a potential source of inoculum, isolations were made from attached dry, brown inflorescences that were sampled from a bay tree. Just beneath the same bay tree a number of loose bay flowers that were resting on a grand fir branch where a small amount of green needle shedding had been observed were collected and also tested. *P. ramorum* was isolated from the peduncle of flowers collected from the bay tree, but not from the flowers that had fallen on the branch.

To confirm that bay flowers can be infected by *P. ramorum*, bay branches with new inflorescences ranging from tight with the brown "caps" in place to fully open flowers were collected from several trees at our experimental site near Los Gatos, CA on February 6, 2007. Ten small shoots with leaves and flowers were then removed from these branches and used for our inoculation test. A bay isolate was not available so we used an isolate from tanoak (NA1 lineage, accession number 2018.1, all isolates reported here are stored at Washington State University). The flowers were carefully dipped for 5 seconds in spore suspension (304,500 zoospores/ml) to avoid getting suspension on the leaves or leaf scars. Check flowers were dipped in water. The bases of the shoots were placed in water in individual, parafilm-sealed flasks. The flasks were then placed in a covered plastic tub with warm water to maintain high humidity and incubated at 17°C.

After 3 days, brown discoloration was observed on some of the inoculated flowers. After 5 days, isolations were made on corn meal agar amended with ampicillin, rifampicin and pimaricin (CARP) from both checks and inoculated flowers. Flowers from all five of the inoculated branches were positive for *P. ramorum*. Isolations from the check flowers were negative. Additional isolations were made after 12 days, when symptoms included brown peduncles and fully blighted inflorescences. All the inoculated flowers were positive, including the peduncle areas. In addition, while examining the 12-day isolation plates, individual, detached, brown sporangia were observed on the surface of the medium. These apparently had fallen off of the infected inflorescences at the time of plating. All 12-day check isolations were also negative.

With the limited amount of work we have done to date, we have not been able to determine if there is a relationship between the development of pitchy cankers on branches of grand fir and the presence of infected bay flowers. We have isolated *P. ramorum* from inflorescences still attached to bay trees, but not on the detached flowers deposited on the conifer branches. In addition, inoculation studies indicate that bay inflorescences are susceptible to *P. ramorum*. Additional work is in progress to determine if infected bay flowers play any role in the development of the pitchy cankers.

Mistletoe: In 2005, a few white fir (*Abies concolor*) and Douglas-fir Christmas trees were found to have a limited number of *P. ramorum*-infected shoots at another farm near our research site. The infection on these trees was unexpected because they were not adjacent to known hosts of *P. ramorum*. Most of the infected trees were within the drip line of a large black walnut (*Juglans nigra*) tree that was infected with mistletoe (*Phoradendron serotinum* subsp. *macrophyllum*).

Although walnut and mistletoe have not been shown to be hosts of *P. ramorum*, we noticed that pieces of mistletoe that had fallen out of the walnut tree had dark spots on old "flower stalks", leaves, and stems. During May 2006, we collected a number of pieces of mistletoe that had fallen out of the walnut tree. Although no *P. ramorum* was recovered from any of the mistletoe leaf or stem tissue, it was isolated from the base of a blackened inflorescence.

Additional samples of mistletoe were collected in June 2006, using a rifle to shoot twigs down from the tree. Shoots were also removed from clumps of mistletoe that were cut out of the tree during February 2007. There were very few symptoms on any of this material and isolations on CARP from these samples were all negative.

We conducted two in vitro inoculation experiments to confirm the susceptibility of mistletoe to this pathogen. In November 2006, we inoculated healthy mistletoe collected a little north of Fresno, California. Eight shoots were selected for our inoculation test. The shoots were placed in water in individual, parafilm-sealed flasks. Unfortunately, our mistletoe isolate was not producing sporangia at the time, so we used an NA1 genotype tanoak isolate (accession number 2027.1). A spore suspension (283,000 zoospores/ml) was applied to the leaves, stems and fruiting "stalks" on four of the shoots using an airbrush sprayer. Check shoots were sprayed with water only. Shoots were incubated as described previously. The plants were examined after 7 days and isolations were made onto CARP from stem lesions, leaf spots and dark areas on the fruiting "stalks". All isolated plant parts were positive for *P. ramorum*. All isolations from the checks were negative.

In early February 2007, we collected healthy-looking mistletoe from the walnut tree near Los Gatos. This material was inoculated using our original isolate from mistletoe (NA1 lineage, accession number 107-0001). Eight small branches with leaves, berries, and/or flower "stalks" were inoculated by spraying them with a spore suspension containing 170,000 zoospores/ml. Eight similar check branches were sprayed with water. Symptomatic tissues were plated onto CARP 3 and 9 days after inoculation and seven of the eight inoculated branches were positive for *P. ramorum*. There were no symptoms on any of the checks and the pathogen could not be re-isolated.

This report confirms the pathogenicity of *P. ramorum* to *Phoradendron serotinum* subsp. *macrophyllum* and is a first record of this plant species as a host for *P. ramorum.* However, whether the mistletoe became infected when living as a parasite in the walnut tree or when it was debris on the ground below the diseased Douglas-fir and white fir trees, is unresolved. Work is currently in progress to confirm that mistletoe plants in the walnut tree are infected by *P. ramorum* and determine what role they play in the spread of disease to the conifers below this tree. If mistletoe is confirmed to be a host of *P. ramorum*, this may have other implications relating to the spread of this pathogen in a landscape situation.

Key words: *Phytophthora ramorum*, *Phoradendron serotinum* subsp. *macrophyllum*, conifers, bay laurel flowers.

Acknowledgments

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Attraction of Ambrosia and Bark Beetles to Coast Live Oaks Infected by *Phytophthora ramorum*[1]

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Abstract

Sudden oak death, caused by *Phytophthora ramorum* (Werres, de Cock & Man in't Veld), has killed thousands of oaks (*Quercus* spp.) in coastal California forests since the mid-1990s. Bark and ambrosia beetles that normally colonize dead or severely weakened trees selectively tunnel into the bleeding cankers that are the first visible symptoms to appear on infected coast live oaks, *Q. agrifolia* (Nee). The role of these beetles is of interest because once beetles attacked infected trees, median survival was found to decline, from seven to less than three years (McPherson and others 2005). Beetle attacks consistently precede the emergence of sporophores of *Hypoxylon thouarsianum* (Leveille) Lloyd, a decay fungus that structurally weakens infected trees. The dense clusters of ambrosia beetle tunnels can extend greater than 15 cm into the sapwood, impairing water conduction and potentially introducing opportunistic organisms. This research has three principal goals: 1. Quantify beetle responses to *P. ramorum*-infected coast live oaks; 2. Identify the factors that influence beetle attacks on these trees; 3. Evaluate the short and long term effects of beetles on colonized trees.

This study was conducted in protected land of the Marin County Open Space District. In July 2002, we mechanically inoculated 80 mature coast live oaks with *P. ramorum* and wounded another 40 without inoculation as controls for beetle responses to wounds. To prevent beetle attacks on trees, we sprayed half of each group to a height of 2.5 m with permethrin, an insecticide with relatively low toxicity to non-target organisms. Insecticide was applied following inoculation, then twice per year, in February and August, prior to the principal beetle flights. Sticky traps were hung on the insecticide-treated trees to monitor insect landing and beetles were collected from these traps periodically through 2003.

Eighty percent of inoculated trees developed bleeding symptoms. Within 18 months after inoculation, trees exhibited the sequence of symptom progression observed in natural forest infections: bleeding, bleeding plus beetle attacks; and bleeding plus beetle attacks plus emergence of *H. thouarsianum* sporophores. Canker size, defined by bleeding surface area, had a mean value of 0.19 m^2 (standard error = 0.03), with a range from 0.008 m^2 to 1.13 m^2.

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Although beetle attacks were not completely prevented by the permethrin treatment, beetle attacks on sprayed trees were delayed by about two months, compared with the unsprayed inoculated trees. The delay resulted in significantly fewer attacks throughout the year on the sprayed trees (repeated measures ANOVA, $F_{1, 53} = 6.2$, $P = 0.016$).

The two principal variables that were found to significantly influence the responses of beetles to infected trees were the surface area covered by bleeding cankers and the numbers of beetles attacking these infected trees. Canker size measured in April 2003, before beetles had attacked any of the permethrin-treated trees, was a significant predictor of (log-transformed) trap catch for the year ($F_{1,23} = 7.06$, $P = 0.014$). By May, after the effectiveness of the insecticide declined, the number of beetle attacks per tree was the only variable that significantly affected trap catch for the year (repeated measures ANOVA, $F_{1,21} = 32.63$, $P < 0.0001$).

Total trap catch for 2003 was 2,770 beetles, >95% of which were trapped on the inoculated trees. All species showed preference for infected trees. The principal beetle species trapped were (in order of decreasing abundance) *Monarthrum scutellare* (LeConte), *Xyleborinus saxeseni* (Ratzeburg), *Pseudopityophthorus pubipennis* (LeConte), *M. dentigerum* (LeConte), *Scobicia declivis* (LeConte), *Xyleborus californicus* (Wood), and *Gnathotrichus pilosus* (LeConte). Most of these species are sapwood-colonizing ambrosia beetles that are typically associated with recently killed hardwoods. Three of these saprotrophic ambrosia beetles are North American, and both *X. saxeseni* and *X. californicus* are Asian introductions. The oak bark beetle, *P. pubipennis*, feeds primarily in dead oaks and the lead cable borer, *S. declivis*, is a generalist beetle that tunnels into both hardwoods and softwoods. The number of beetles trapped on inoculated trees in 2003 was strongly correlated with advanced disease stage (trees that were attacked by beetles and those that also showed *H. thouarsianum* sporophores) through December 2006 (ordinal logistic regression, $\chi_1^2 = 28.56$, $P < 0.001$).

Despite the transience of the permethrin treatment, the effects of preventing the first beetle attacks for two months have persisted for more than three years. The lower number of beetle attacks on the trees that were treated with insecticide was correlated with lower mortality. By December 2006, three of the permethrin-treated trees had died, compared with nine unsprayed trees. The 12 trees that died had all been extensively colonized by beetles and all but one had *H. thouarsianum* sporophores in the areas where beetles attacked early in 2003.

The relationship between canker surface area and trap catch prior to beetle attacks is probably due to beetles responding to the production of volatile host- and/or host/pathogen-derived attractant compounds. Greater trap catch in 2003 was positively correlated with advanced disease status more than three years later, which suggests that the attraction of beetles to bleeding trees, as detected by trap catch, predicted the intensity of beetle attacks, and subsequently, the expression of disease in the trees. Further support for the role of volatile cues in the beetle response comes from the observation that beetles attacked some of the inoculated trees three months after they were inoculated, which was four months before the appearance of bleeding confirmed that they were infected. Once beetles had begun attacking the insecticide-treated trees, their production of aggregation and sex pheromones (for example, Wood 1982; Paine and others 1997) was probably the principal behavioral cue attracting additional beetles to infected trees.

This work provides strong support for the hypothesis that beetles that tunnel deeply into the xylem of coast live oaks infected by *P. ramorum* accelerate disease progression and contribute to bole failure of living trees (Svihra and Kelly 2004). Where individual coast live oaks may be capable of limiting the expansion of cankers, beetle attacks on these cankers may irreversibly change the course of the disease. Tunnels provide entry for decay fungi, as well as pathogens, that are otherwise excluded from access to the sapwood resource. Research into

the relative importance of fungi and beetles in *P. ramorum* infection of coast live oaks is ongoing.

Key words: *Phytophthora ramorum, Quercus agrifolia*, permethrin.

Acknowledgments

We wish to thank Dr. David Rizzo for providing *P. ramorum* cultures and Dr. Donald Bright for identifying the beetles. The field and laboratory assistance of Gabriela Ritokova and Andrew Nelson were critical. The United States Department of Agriculture-Forest Service, Pacific Southwest Research Station, provided funding for this study through grant number 02-CA-11272138-060. Marin County Open Space District provided access to research sites.

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Susceptibility to *Phytophthora ramorum* in California Bay Laurel, a Key Foliar Host of Sudden Oak Death[1]

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Abstract

Sudden oak death, caused by the water mold *Phytophthora ramorum*, is a plant disease responsible for the death of hundreds of thousands of oak and tanoak trees. Some foliar hosts play a major role in the epidemiology of this disease. Upon infection by *P. ramorum*, these foliar hosts express non-fatal leaf lesions from which large amounts of inoculum can be produced and spread to neighboring host individuals, including oak species. *Umbellularia californica* (California bay laurel) may be one of the most important foliar hosts of sudden oak death due its observed ability to produce inoculum and its high abundance in the woodlands of coastal California. While previous research on susceptibility to *P. ramorum* in *U. californica* has shown significant variability among trees, with more resistant individuals in northern areas of its range, little is known about the causes or extent of this variability. Here, we ask three research questions: (1) How does susceptibility vary among *U. californica* individuals and *P. ramorum* isolates? (2) Are *U. californica* phenotype and genotype related to susceptibility? (3) What factors influence disease expression in nature?

We conducted lab susceptibility trials on detached leaf samples and assessed field symptom levels for 97 *U. californica* trees from 12 plots, four from each of three regional clusters, in a 275 km^2 area in Sonoma County, California. In each plot, field disease expression was quantified using 90 second timed counts of the number of symptomatic leaves. For each tree,

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leaves were collected for laboratory analysis of AFLP (amplified fragment length polymorphism) molecular markers, leaf toughness, water content, and susceptibility. Susceptibility trials were conducted by inoculating leaves with two *P. ramorum* isolates and scoring resulting lesion size. Within a GIS, latitude and longitude, elevation, topographic moisture index, and annual precipitation were calculated for each plot. In addition to the 97 Sonoma county trees, leaves for susceptibility trials were also collected from five trees from a high oak mortality reference site in Marin County.

We found that susceptibility varied significantly among *U. californica* trees, with a five fold difference in lesion size. The Marin County individuals developed significantly larger lesions, but significant differences were not found among the 12 plots in Sonoma County. The phenotypic trait of leaf area was significantly related to lesion size, where bigger leaves produced bigger lesions. The two different isolates produced similar sized lesions.

We found variability in lesion size produced on detached leaves was significantly related to six AFLP markers (loci were screened using a series of one-way ANOVAs; each of the six loci were significantly related to lesion area at $P < 0.05$), suggesting a genetic basis to resistance. Molecular marker analysis also revealed genetic structure in this species was partitioned significantly within plots, among plots, and among plot clusters, but the greatest diversity levels were found within plots. Variation in field symptom levels was significantly different among plots and primarily correlated with environmental site conditions, including longitude, topographic moisture index, mean precipitation, and mean daily temperature minimum. There was no relationship between lesion size produced in the laboratory and symptomatic leaf count in the field, suggesting that local environmental conditions influence disease expression in nature more than genetic or phenotypic host factors, at the scale of this study.

This work demonstrates that susceptibility to *P. ramorum* in *U. californica* depends on genetic, phenotypic, and environmental characteristics, as well as *P. ramorum* isolate virulence, and provides useful information for predicting spread risk among *U. californica* and onto oak trees.

A more detailed account of this work can be found in the subsequent journal publication: Anacker, B.L.; Rank, N.E.; Daniel Hüberli, D.; Garbelotto, M;, Gordon, S.; Harnik, T.; Whitkus, R.; Meentemeyer, R. 2007. Susceptibility to *Phytophthora ramorum* in a key infectious host: landscape variation in host genotype, phenotype, and environmental factors. New Phytologist. doi: 10.1111/j.1469-8137.2007.02297.x.

Key words: *Umbellularia californica*, California bay laurel, *Phytophthora ramorum*, sudden oak death, disease susceptibility, sporangia, amplified fragment length polymorphism (AFLP).

Human Activity and the Spread of *Phytophthora ramorum*[1]

J. Hall Cushman,[2] Michelle Cooper,[3] Ross K. Meentemeyer,[4] and Shelly Benson[5]

Abstract

Increasing numbers of studies are finding that humans can facilitate the spread of exotic plant species in protected wildlands. Hiking trails commonly serve as conduits for invaders and the number of exotic plant species occurring in protected areas is often correlated positively with visitation rates. Despite such evidence linking human activity to the spread of exotic plants, few studies have addressed this possibility for plant pathogens.

Over the past 4 years, we have been evaluating the role that humans play in promoting the spread of *Phytophthora. ramorum* and the disease it causes. Our previous research has suggested that human activity is hastening the spread of *P. ramorum* in northern California: the pathogen was more commonly found in soil on hiking trails than from soil in adjacent areas off trails; public lands open to recreation had higher proportions of diseased host trees than private lands; and the chance that host trees were infected by *P. ramorum* increased as the density of human populations increased in the surrounding area. Collectively, these data suggest that human activity can inadvertently disperse *P. ramorum* throughout the landscape, further spreading the pathogen into already infested areas and introducing it into previously uninfested areas.

More recently, we have conducted additional studies that further link two forms of human activity – hiking and mountain biking – to the dispersal of *P. ramorum*. First, at a nature preserve in Sonoma County, we have shown that hikers can disperse *P. ramorum* in soil on their shoes at least 60 to 100 m into areas that lack local sources of inoculum. Second, we found that 5 to 10 percent of the visitors entering a recreational area in Marin county had the pathogen in soil on their shoes and tires, and 20 to 30 percent carried it out with them. Although hikers and mountain bikers did not differ significantly in the capacity to transport *P. ramorum*, there was a trend indicating that during dryer conditions, the further a person traveled along a trail, the more likely they were to pick up and transport the pathogen. In addition, although our data suggest that humans can serve as effective dispersal agents, the temporal window for doing so is constrained, as the pathogen could not be cultured from soil on hikers' shoes after 24 hours, although this time was extended to at least 72 hours if the soil on hiking shoes was kept moist. These results suggest that human dispersal of *P. ramorum* may be limited to certain kinds of situations: further spread of the pathogen in already infested

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areas or instances in which visitors move rapidly from one region to another, especially when hiking shoes or mountain bikes have been stored in moist conditions.

In summary, our research suggests that there may be conflicts between human activities and disease spread, and that efforts to address this epidemic may require aggressive management, which may be logistically and politically challenging to implement.

Key words: *Phytophthora ramorum*, human dispersal, human population density, recreation.

Increasing Distance from California Bay Laurel Reduces the Risk and Severity of *Phytophthora ramorum* Canker in Coast Live Oak [1]

Tedmund J. Swiecki [2] and Elizabeth A. Bernhardt [2]

Abstract

Foliar infections in California bay (*Umbellularia californica*) are the most important known source of inoculum contributing to *Phytophthora ramorum* canker in coast live oak (*Quercus agrifolia*). This research addressed the question whether there is a "safe" distance between California bay and coast live oak beyond which the risk of disease is acceptably low. We quantitatively evaluated bay cover and other factors in the neighborhoods around 247 coast live oaks in long term research plots in mixed hardwood forests where *P. ramorum* canker has been prevalent since 2000. Both the risk and severity of *P. ramorum* canker decreased as the minimum distance between California bay foliage and the oak trunk increased. Disease risk and severity were greatest at bay foliage-oak trunk distances of 1.5 m or less and were minimal at a distance of 10 m or more. Bay cover within 2.5 m of the trunk was a stronger predictor of disease risk and severity than the minimum bay-trunk distance. These results suggest that removing bay from within 2.5 m of the trunk of a susceptible oak will greatly reduce, but not eliminate, the risk of disease. For some oaks with *P. ramorum* canker, the presence of disease symptoms could not be readily explained by proximity to bay, but large amounts of poison oak (*Toxicodendron diversilobum*) vines climbing in the oak canopy or in adjacent trees appeared to be the most likely source of inoculum. Based on timed counts of symptomatic bay leaves repeated at intervals between fall 2005 and fall 2006, bay foliar infection levels were minimal in fall and peaked in late spring and summer. Counts of infected leaves in fall 2005 were not correlated with counts from the same trees in either spring/summer 2006 or fall 2006, but spring/summer 2006 counts were correlated with fall 2006 counts.

Key words: *Umbellularia californica*, *Quercus agrifolia*, disease risk, disease severity, cover, clearance.

Introduction

On coast live oak (*Quercus agrifolia*), *P. ramorum* canker or sudden oak death (SOD) exhibits a patchy distribution both within its range in California and within affected stands (Rizzo and others 2005). Some of this patchiness may be related to the length of time that has elapsed since the pathogen was introduced into the stand

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(Rizzo and others 2005; Swiecki and Bernhardt, in press). However, even within stands that have been heavily infested with *P. ramorum* for at least 7 years, SOD has not become uniformly spread throughout the stands (Swiecki and Bernhardt 2006), which suggests that the epidemiology of the disease is strongly influenced by factors operating at a localized spatial scale.

Since 2000, we have been studying disease risk and progress in 150 long-term research plots in areas where *P. ramorum* canker is prevalent (Swiecki and Bernhardt 2006). Our analyses indicate that both tree- and plot-level factors are significant predictors of *P. ramorum* canker for coast live oak. A number of characteristics that are seen exclusively or primarily in relatively vigorous, fast growing trees are significantly associated with high disease risk. In addition, the presence and abundance of California bay (*Umbellularia californica*) within plots was identified as a significant plot-level predictor of disease risk in our initial data analyses (Swiecki and Bernhardt 2001). Several related variables, including counts of bay trees within the 8 m radius plot and plot bay cover are significant predictors of SOD risk (Swiecki and Bernhardt 2004), showing that disease risk increases with increasing bay density and cover within 8 m of a coast live oak.

Davidson and others (2002, 2005) showed that *P. ramorum* infects and sporulates abundantly on bay foliage, but does not sporulate on coast live oak. The amount of *P. ramorum* inoculum dispersed from bay canopies decreased rapidly as the distance from the bay canopy source increased from 0 to 5 m or beyond (Davidson and others 2005). Tjosvold and others (2006) did not detect *P. ramorum* propagules more than 1 m away from infected rhododendron source plants, and infection of rhododendron trap plants was not observed more than 0.5 m from infected source plants.

Taken together, these studies indicate that bay foliage closest to a host oak is likely to make the largest contribution to disease risk. However, the studies do not allow us to determine a minimum "safe" bay foliage-oak distance for purposes of disease management. This study was undertaken to determine whether it is possible to specify a bay foliage-oak distance beyond which the risk of disease is reduced to acceptably low levels.

Methods

Study Sites and Plots

The plots used for this study were established in September 2000 for a case-control study on factors influencing development of SOD (Swiecki and Bernhardt 2001). Plots were established in mixed hardwood forests where *P. ramorum* symptoms were prevalent on coast live oak. The study locations were in Marin (nine locations) and Napa (one location) counties.

At each study location, we established circular 8 m radius (0.02 ha) fixed-area plots, each of which was centered at a coast live oak tree. The tree-centered plots were spaced approximately 25 m apart. Trees in the plots were evaluated annually in September of 2000 through 2006 for symptoms of *P. ramorum* canker and other indicators of tree health (Swiecki and Bernhardt 2006). For this study, overall *P. ramorum* symptom status and estimated girdling due to *P. ramorum* cankers were evaluated as the primary disease outcomes.

Phytophthora ramorum symptom status was visually assessed using the following scale: (0) no symptoms; (1) early symptoms: bleeding cankers only; (2) late symptoms: cankers plus *Hypoxylon thouarsianum* sporulation and/or beetle boring; (3) dead as result of *P. ramorum* infection. The disease status of some symptomatic trees was confirmed by isolating the pathogen from bark tissue pieces sampled from the canker margins. *Phytophthora ramorum* was the only *Phytophthora* sp. recovered from cankers at all of the study locations.

The percentage of the oak main stem that was girdled by *P. ramorum* cankers was estimated visually, based on bleeding, bark characteristics such as obvious necrosis or cracking, and, in some trees, limited chipping of bark to expose the canker margins. The overall girdling rating was derived by estimating the extent of all cankers in the lower 2 m of the bole and combining the affected percentage of the circumference as if all cankered areas were on the same stem cross section. Cankers at different heights along the stem increase the girdling rating only if they are horizontally offset around the stem circumference. We used the following 0 to 6 scale, the intervals of which are pretransformed using the arcsine transformation, to estimate the percent of stem circumference girdled: 0 = no girdling seen; 1 = <2.5 percent girdled; 2 = 2.5 to <20 percent girdled; 3 = 20 to <50 percent girdled; 4 = 50 to <80 percent girdled; 5 = 80 to <97.5 percent girdled; 6 = 97.5 to 100 percent girdled or tree dead due to *P. ramorum*.

Tree Selection

Coast live oaks were selected from the study plots to represent cases (trees with SOD symptoms) or controls (trees lacking SOD symptoms). The symptom status of individual trees could be determined with a high degree of reliability because trees had been observed for disease symptoms and disease progress annually between 2000 and 2006. All symptomatic coast live in the study plots, except for those with ambiguous disease symptoms, were selected as cases.

Potential controls included all trees in the plots that were free of *P. ramorum* canker symptoms over the previous 7 years. In selecting controls, we eliminated trees that had tree characteristics that previous models have shown to be associated with low disease risk (Swiecki and Bernhardt 2001, 2004). These included trees that were almost completely overtopped (low sky exposed canopy values), had very low ratings for unweathered tissue in bark fissures, and/or were in severe decline due to agents other than *P. ramorum*.

Trees were selected based on existing data sets to avoid potential bias. Preselected trees were rejected in the field only if major structural failures had occurred in either the selected oak or nearby bays and bay neighborhood prior to tree failure could not be reliably assessed. In all, 247 coast live oak trees were included in this study: 36 percent were asymptomatic, 16 percent had early symptoms, 23 percent had late symptoms, and 24 percent had been killed by *P. ramorum*.

Evaluation of California Bay Laurel Around Oaks

We estimated bay cover within concentric rings centered around each oak tree included in the study. The rings were based on the following distances from the oak

trunk: <2.5 m, 2.5 to 5 m, 5 to 10 m, and 10 to 20 m. Each distance ring was divided into four 90 degree arcs centered at each of the cardinal compass directions. Within the three innermost rings, we estimated the bay cover in each quarter arc of the ring using the following quartile scale: 0 = no bay cover; 1 = 1 to 25 percent bay cover; 2 = 26 to 50 percent bay cover; 3 = 51 to 75 percent bay cover; 4 = more than 75 percent bay cover. For the 10 to 20 m distance ring, only bay presence or absence was noted. Bay cover was assessed between October 2005 and July 2006.

We used an angle gauge with an attached high intensity green laser pointer to project vertical lines into the canopy to help define the edges of distance rings and arcs. A hand-held Leica Disto™ laser was used to measure distance to the oak trunk. We also noted the presence, location, and amount of other foliar hosts of *P. ramorum* in the immediate neighborhood that might serve as alternative sources of inoculum, such as tanoak (*Lithocarpus densiflorus*) or poison oak (*Toxicodendron diversilobum*).

We assessed bay foliar infection level in 106 patches of bay canopy around 37 of the coast live oaks in the study. Contiguous patches of bay foliage, arising from either a single bay stem (47 zones) or multiple (2 to 19) stems were mapped based on distance and azimuth relative to the oak. We used 45-second timed counts of symptomatic leaves to assess foliar disease levels in the mapped bay zones in September and October 2005. Recounts of foliar symptoms in the same bay zones were made between late May and early August 2006 and again in September 2006; a small subsample was also recounted in January 2006. All counts were made by the same observer for all trees and all sampling dates.

Statistical Analyses

We used JMP® statistical software (SAS Inc., Cary NC) for data analysis. Unless otherwise indicated, effects or differences are referred to as significant if $p \leq 0.05$. We used analysis of variance (F-tests) or t-tests to compare means of continuous variables. For ordinal variables such as bay cover percentage ratings, the nonparametric Wilcoxon rank sum test was used to test the significance of differences. Differences between medians were tested using the nonparametric median test. Effects of sampling date and other variables on bay symptom counts were tested using repeated measures analysis of variance. We used linear regression to test for correlations between continuous variables. The nonparametric Spearman test was used to test for correlations between pairs of categorical variables. The square root transformation was applied to bay foliar symptom counts prior to analysis.

Recursive partitioning was used to develop models and investigate interactions between predictors. Recursive partitioning splits data in a dichotomous fashion, with each partition chosen to maximize the difference in the responses between the two branches of the split. We also developed logistic regression models to examine the effects of factors on the binary disease outcome (tree is diseased, in other words, a case) and used generalized linear models to test relationships between various predictor variables and the girdling rank outcome.

Results

Minimum Distance to Bay Foliage

California bay was well-distributed throughout the mixed hardwood forests at the study locations. Only six of the coast live oak trees in the study (2.4 percent) did not have bay present within 20 m of the trunk. Figure 1 shows that the distributions for minimum distances from bay foliage to the oak trunk differed for coast live oaks with and without *P. ramorum* canker symptoms. Although both distributions are strongly left-skewed, the mean and median bay foliage-oak trunk distances were significantly greater in the controls (mean 5.7 m, median 3.3 m) than in the cases (mean 1.3 m, median 0 m).

Poison oak is a known *P. ramorum* host, although inoculum production on this host has not been studied. Overall, five cases (three of which were dead in 2006) had substantial amounts of poison oak climbing in their canopies and three had canopy-level poison oak in adjacent trees at distances of 2.5 m or less from the trunk. Among controls, only one had poison oak climbing in the canopy and no others had canopy-level poison oak within 2.5 m of the trunk. All of the cases with bay foliage-oak trunk distances greater than 10 m either had extensive amounts of poison oak climbing in their canopies (fig. 1) or were within 7.5 m of such trees.

We used recursive partition analysis to more closely examine the relationship between the minimum distance from bay foliage and the presence of *P. ramorum*

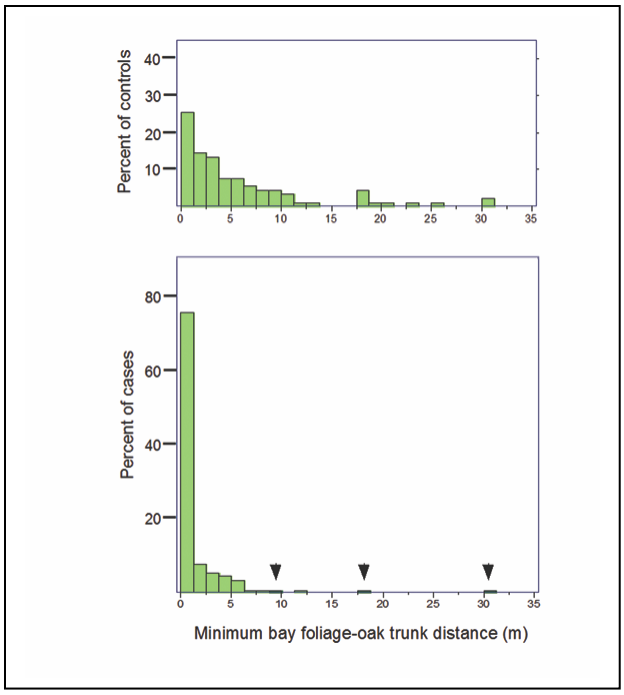

Figure 1—Minimum distance (m) between nearest bay foliage and coast live oak trunks for oaks without (top graph, n=90) or with (bottom graph, n=157) *P. ramorum* canker symptoms. Arrows in the bottom graph indicate three trees with extensive poison oak growing in the canopy (shaded bars).

canker symptoms in coast live oak. The greatest difference in both percent infection and in average *P. ramorum* canker girdling rank was achieved by partitioning at a minimum bay foliage-oak trunk distance of 1.5 m. For oaks with a minimum bay foliage-oak trunk distance of less than 1.5 m, *P. ramorum* canker incidence was 83 percent and average girdling rank was 3.8 (nearly 80 percent girdling). In oaks with a bay foliage-oak trunk distance greater than or equal to 1.5 m, *P. ramorum* canker incidence was 33 percent and average girdling rank was 1.3 (less than 20 percent girdling). Furthermore, coast live oaks with bay foliage within 1.5 m of the trunk were more likely to have advanced disease symptoms (late or dead) than oaks for which the bay foliage-oak trunk distance was greater than 1.5 m.

We also used recursive partition models to examine the relationship between *P. ramorum*-related mortality and minimum bay foliage-oak trunk distance. A minimum bay foliage-oak trunk distance of 0.5 m provided the greatest difference in levels of mortality associated with *P. ramorum*. Based on a single variable logistic regression model (model $p<0.0001$) for mortality, oaks with bay foliage within 0.5 m of the trunk were almost nine times more likely to have been killed by *P. ramorum* than trees with greater bay foliage-oak trunk distances (odds ratio = 8.7; 95 percent confidence interval = 4.2 - 20).

Figure 2 illustrates how the incidences of *P. ramorum* symptoms, *P. ramorum*-related mortality, and disease severity (based on girdling rating) decrease with increasing minimum bay foliage-oak trunk distance. The only symptomatic oak in the >10 m minimum bay foliage-oak trunk distance class (n = 15) had a minimum bay foliage-oak trunk distance of 12 m and had canopy-level poison oak present 7.5 m from the trunk. Both the incidence of *P. ramorum* canker and average girdling rank decreased as the minimum bay foliage-oak trunk distance increased, but *P. ramorum*-related mortality did not change significantly for distance classes beyond 0 m (fig. 2).

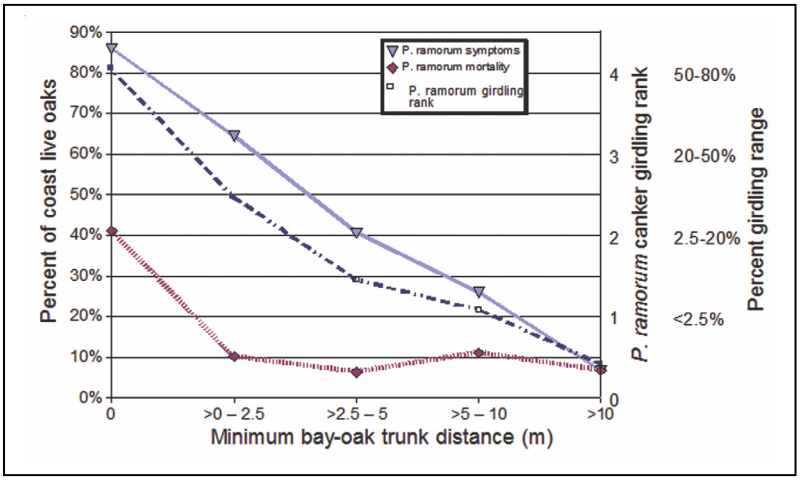

Figure 2—Percent of coast live oaks with *P. ramorum* symptoms and mortality due to *P. ramorum* (left scale), and average *P. ramorum* girdling rating (right scale) by minimum bay foliage-oak trunk distance class. Sample sizes for the distance classes from left to right are 107, 59, 32, 27, and 15. Trees with poison oak growing in the canopy or in adjacent trees within 1.5 m of the oak trunk are omitted.

Bay Cover Within Distance Rings

Analysis of bay cover data for the various distance rings around the cases and controls is complicated by correlations between these variables. The mean bay cover ratings from each distance ring show significant positive correlations with all other rings, although the highest correlations are seen between adjacent distance rings (table 1). In addition, minimum bay foliage-oak trunk distance is negatively correlated with bay cover ratings for each of the distance rings, although the strongest correlations are seen for the distance zones closest to the oak trunk (table 1). These correlations are related to the overall spatial distribution of bays around coast live oaks and the fact that many of the bay canopies were large enough to span multiple distance rings.

Table 1—Spearman's rho rank correlation coefficients for pairwise comparisons between bay distance and cover variables. All correlations shown are significant at p<0.0001

	0-2.5 m bay cover	2.5-5 m bay cover	5-10 m bay cover	10-20 m bay presence
Minimum bay foliage-oak trunk distance	-0.9095	-0.8047	-0.5744	-0.4004
0-2.5 m bay cover		0.8644	0.6113	0.3445
2.5-5 m bay cover			0.7626	0.3801
5-10 m bay cover				0.5764

One consequence of the strong correlations is confounding of some variables: combinations that are needed to differentiate between effects of certain variables either are lacking or represented by too few points to be statistically meaningful. In particular, our ability to differentiate between the effects of bay cover within 2.5 m of the oak trunk and bay cover between 2.5 and 5 m from the oak trunk is limited because the bay cover within these two zones is highly concordant in this data set.

As shown in figure 3, the average bay cover ratings for cases are significantly greater than those of controls for all distance rings. These significant differences persist if oaks with minimum bay foliage-oak trunk distances of less than 0.5 m are omitted, although the significance level of the 10-20 m zone is slightly decreased (p=0.011, Wilcoxon rank sum test). Although the relative differences in bay cover between cases and controls become smaller as the distance from the oak increases (fig. 3), it is difficult to separate the effects of bay cover in the different zones due to the high level of correlation between the distance classes (table 1).

We used recursive partitioning to investigate the relative ability of bay cover variables to predict disease outcomes. *P. ramorum* canker girdling rank, which takes both disease incidence and disease severity into account, was used as the disease outcome. Oaks with canopy-level poison oak within 1.5 m of the trunk were omitted, although the first two splits of the recursive partition model are nearly the same if these trees are included. Using the four variables in table 1, the initial partition was based on the average bay cover rating within 2.5 m of the oak trunk (table 2). The

next two splits were based on bay cover in more distant rings. The minimum bay foliage-oak distance was not used as a splitting criterion until the fourth partition (table 2).

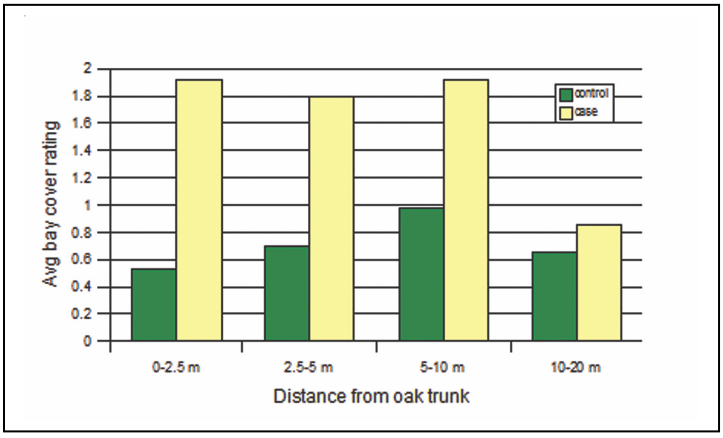

Figure 3—Average California bay cover ratings in distance rings around coast live oak controls and cases. For distance rings from 0 to 10 m, ratings were made using the quartile scale (maximum rating=4); for the 10-20 m ring, only presence (1) or absence (0) was scored. All differences between cases and controls are significant at $p<0.0001$ according to a two-tailed t-test (distance rings from 0 to 10 m) or Wilcoxon rank sum test (10-20 m distance ring).

Table 2—Recursive partition model for the *P. ramorum* girdling rank disease outcome. Candidate predictors were the four variables shown in Table 1. Overall model R^2=0.334. Trees with poison oak within the canopy or at canopy level within 1.5 m of the trunk were excluded from the data set. Note that girdling ranks are non-linear (see methods)

Predictor variable *cutting value*			**n**	**Mean *P. ramorum* girdling rank**	***P. ramorum* incidence (percent)**
bay cover <2.5 m *< 0.775*	bay cover 5-10 m *<0.75*		37	0.38	13.5
	bay cover 5-10 m *≥ 0.75*		72	1.81	45.8
bay cover <2.5 m *≥ 0.775*	bay cover 2.5-5 m *≥ 1.775*		86	4.27	90.7
	bay cover 2.5-5 m *< 1.775*	Min bay foliage-oak trunk dist *< 0.5 m*	37	3.59	81.1
		Min bay foliage-oak trunk dist *0.5 m*	8	1.75	62.5

This model indicates that bay cover within 2.5 m of the oak trunk is the best single predictor of *P. ramorum* canker incidence and severity in these trees. Oaks with 25% cover or more in this zone showed the highest disease incidence and severity. However, higher levels of bay cover in further distance zones (to at least 10 m) also tend to increase disease incidence and severity, although the confounding of the data does not allow us to derive a robust estimate of the disease risk associated with bay cover in the farther zones.

P. ramorum Foliar Symptoms on Bay

Bay foliar symptoms were generally distributed in a nonuniform fashion within individual bay zones and among the bay zones surrounding a given oak. In general, symptomatic bay leaves were more common in the generally shaded lower portions of the canopy than in the more exposed uppermost portions. In addition, symptoms were generally less common on open-grown trees, especially if they were relatively small and/or appeared water-stressed (leaves relatively small and somewhat chlorotic).

Figure 4 shows how bay foliar symptom counts varied through a single year for 12 bay zones at one location. The general pattern of seasonal variation was similar for all bay zones: counts were at or near minimum values in September/October and at maximum values in late spring/early summer. Reductions in the number of symptomatic leaves that occurred over the summer were due to early dehiscence of infected leaves. In some locations, many symptomatic leaves had become chlorotic by early May 2006, and these leaves had dropped by September. Bay zones within a given location varied considerably with respect to the maximum and minimum counts observed over the year and the timing of increases and decreases in infected leaf counts (fig. 4).

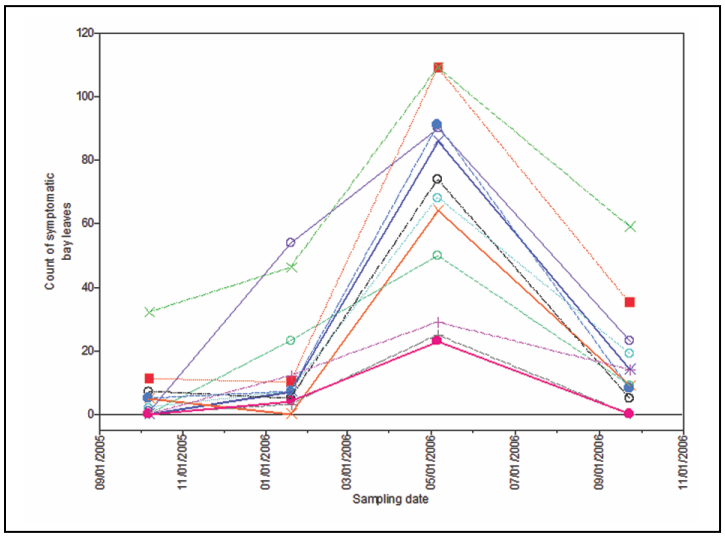

Figure 4—Number of infected bay leaves counted in a 45 second search period for 12 bay zones at location 5 assessed on four dates between September 2005 and October 2006. Connected points represent counts in the same bay zone. Within each graph, zones with the same symbol type are located around the same oak.

To determine how bay foliar symptom counts varied between locations, we selected the six locations for which midseason counts were made in the May-June interval, to reduce variation associated with the timing of the late spring-early summer evaluation. Repeated measures analysis of variance showed that symptomatic leaf counts varied significantly over time ($p<0.0001$), and by study location (time × location interaction $p<0.0001$). Counts did not differ significantly based on the number of trees within a bay zone.

Symptomatic bay leaf counts of individual bay zones made in fall 2005 were not significantly correlated with counts made in fall 2006 (n=106), January 2006 (n=15), May-June 2006 (n=80), or July-August 2006 (n=33). However, symptomatic bay leaf counts from May-June 2006 and September 2006 were significantly correlated (n=80, $p<0.0001$, $R^2=0.411$; square root-transformed counts) as were July-August 2006 and September 2006 counts (n=33, $p=0.0011$, $R^2=0.294$; square root-transformed counts).

We also calculated an overall average count for all bay zones around each of the 37 coast live oaks included in this portion of the study. These averages are analogous to averages for a plot centered around each oak. As was seen for the correlations on individual bay zones, average counts of symptomatic bay leaves for zones surrounding individual oaks were not correlated between September 2005 and September 2006 (n=37) or between September 2005 and May-June 2006 (n=28), but May-June 2006 counts were significantly correlated with September 2006 counts ($p=0.0007$, $R^2=0.365$, n=28 for square root-transformed means of counts). The average symptomatic bay leaf counts for the zones surrounding these oaks were not significant predictors of either the binary disease outcome or the *P. ramorum* canker girdling rank outcome.

Discussion

Bay Variables that Influence Disease Risk

For the coast live oaks in this study, both the risk of *P. ramorum* infection and the severity of *P. ramorum* canker symptoms increased as the horizontal distance between bay foliage and the oak trunk decreased. The risk of disease, severe symptom development and mortality were highest at bay foliage-oak trunk distances between 0 and 1.5 m. This distance is similar to the range of splash dispersal of *P. ramorum* observed by Tjosvold and others (2006) from infected container-grown rhododendrons. Most propagules of other *Phytophthora* species (Timmer and others 2000) and other pathogens (Grove and Biggs 2006) dispersed by splashing from plant surfaces impact within 1 to 2 m of the inoculum source in the absence of high winds.

Where bay foliage is present within about 1.5 m of the oak trunk, *P. ramorum* inoculum can impact the trunk via droplets splashed from infected leaves or water that directly runs off bay foliage and drips on the trunk. These processes are likely to deliver much greater amounts of inoculum to the oak trunk than would be deposited via wind-blown droplets. Davidson and others (2005) showed that the highest numbers of *P. ramorum* propagules dispersed under natural conditions from infected bay canopy at a forest edge were found directly under bay canopy. Progressively fewer propagules were detected at distances of 5, 10, or 15 m from bay canopy. These greater distances involve dispersal of droplets by wind across unobstructed airspace. For splash dispersed inoculum, the decline in inoculum concentration with

increasing distance from the source generally follows power law or exponential models (Ahimera and others 2004, Huber and others 1996), which are characterized by steep declines in inoculum concentration within the first meter from the source.

Given that the highest risk and severity of *P. ramorum* canker were associated with short bay foliage-trunk distances where inoculum concentrations would be quite high, we conclude that relatively high *P. ramorum* inoculum concentrations are typically required to initiate severe symptom development in most coast live oaks. This conclusion is further supported by the fact that bay cover within 2.5 m of the oak trunk is a stronger predictor of disease risk and severity in coast live oak than is the minimum bay foliage-oak trunk distance. Because bay cover ratings are related to bay leaf area, bay cover is more directly related to potential levels of inoculum production than is bay foliage-trunk distance.

Although severe disease and mortality due to *P. ramorum* is most commonly associated with high amounts of bay cover adjacent to the oak trunk, disease sometimes develops in trees that do not fit this profile. This suggests that alternative sources of inoculum, such as poison oak, may be important in some situations. Alternatively, some trees may be so highly susceptible to *P. ramorum* infection that small amounts of inoculum can initiate successful and sometimes lethal infections.

Foliar Infection Levels in Bay

Because disease risk in coast live oak appears to be highly correlated with the level of inoculum produced on bay close to the oak, we expect that levels of foliar infection in bay would correlate with disease risk in a prospective study that examines the initiation of disease in healthy oaks. However, since this is a retrospective study, we were unable to observe *P. ramorum* infection levels in bay that existed at the time the oaks became infected. If bay foliar infection levels within specific patches of bay canopy were highly correlated from year to year, infection levels measured in any given year might still be a useful predictor of *P. ramorum* canker risk. However, our data on bay foliar infection levels failed to show either clear year to year correlations in foliar symptom levels or any significant relationship between foliar symptom levels and disease on adjacent oaks.

Bay foliar infection levels in patches of bay foliage were correlated within a single year, and showed a decline in infection level over time as symptomatic leaves dropped. This indicates that foliar symptom counts need to be made over a sufficiently short time interval to minimize variation due to seasonal loss of symptomatic leaves.

Rank and others (these proceedings) have shown significant correlations between bay foliar counts for individual trees made in late spring/early summer of 2004 and 2005, when symptom levels are near their maximum. Their data are not directly comparable to ours due to differences in assessment methodology, characteristics of the study locations, and timing of the assessments. Although foliar infection levels in patches of bay foliage can be correlated over relatively short time intervals, the correlation is likely to break down over successively longer time intervals. Especially in stands that have high levels of *P. ramorum*-related mortality, the change in microclimate over time due to the loss of tree canopy could result in substantial changes in the potential for bay foliar infection.

Management Considerations

Due to the confounding of several variables, we are only partially able to address the question as to what constitutes a "safe" bay foliage-oak distance with respect to the risk of SOD. While it is probably possible to prevent nearly all *P. ramorum* infections in coast live oak by clearing all bay within 10 m of the oak trunk, this strategy is probably best suited for protecting a relatively few individual high-value trees at a given site. In many locations, obtaining 10 m of clearance from all susceptible oaks would require nearly complete removal of bay from a stand, which may not be financially feasible or consistent with other forest management objectives or landowner preferences.

If both disease incidence and severity are considered, bay foliage located within about 2.5 m of the trunk pose the greatest risk to coast live oak. At minimum, removal of bay foliage from this zone should substantially decrease the risk of both disease and mortality due to *P. ramorum*. Bay present at distances between 2.5 and 10 m may also increase disease risk, especially if it is located in the direction of prevailing storm winds (Swiecki and Bernhardt 2007). However, our current data set lacks examples of situations where the no bay cover is found within 2.5 m of the oak trunk but high bay cover levels are found beyond this point, so it is not possible to quantify the disease risk associated with bay foliage that is exclusively found in the 2.5 to 10 m range. For purposes of management, each doubling of clearance distance quadruples the area that needs to be cleared, so incremental reductions in disease risk need to be weighed against the increased cost of developing additional clearance.

We recently initiated a study in which selective bay removal and pruning is being used to create localized areas free of bay foliage near oak trunks. Based on the analyses presented here, we used the following prescription for selective bay removal around individual oaks. We believe this represents a reasonable balance between minimizing disease risk and the cost of bay removal.

- Establish a minimum of 2.5 m of horizontal clearance between bay foliage and the oak trunk, including removal of small understory bay seedlings and saplings within at least 2 m of the oak trunk.

- Where feasible with a minimum of additional bay removal, extend the clearance to 5 m, especially toward the normal storm wind direction (Swiecki and Bernhardt 2007)

- Where it is difficult to completely remove bay in the 2.5 to 5 m distance range, remove low bay canopy by pruning low branches.

- Cut stems of poison oak that are climbing into the canopy of the oak or adjacent trees to provide at least 2.5 m of horizontal distance between canopy-level poison oak and the oak trunk.

Implementing this prescription around an individual oak should significantly reduce the likelihood that the oak will develop or be killed by *P. ramorum* canker, but may not be sufficient to completely prevent disease in all treated trees. In addition, it may not be feasible to implement this prescription for all trees in a stand, especially where very large bays are present. This prescription is most appropriate for reducing

potential disease impacts in stands where adequate clearances can be established around asymptomatic oaks by removing and/or pruning relatively small-diameter bays. The new study we have initiated will evaluate the efficacy of this prescription.

Acknowledgments

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Dissemination of Aerial and Root Infecting *Phytophthoras* by Human Vectors[1]

J.F. Webber[2] and J. Rose[2]

Abstract

Two new *Phytophthora* pathogens, *Phytophthora kernoviae* and *P. ramorum*, have recently established in parts of the U.K. They are most prevalent in the south west of England where they cause intense episodes of foliar blight and dieback on both ornamental and naturalised rhododendron such as *Rhododendron ponticum*, but both also cause lethal stem cankers on a range of broadleaved trees. Patterns of disease spread suggest that both pathogens could be spread over longer distances by vertebrate movement. People and animals frequently walk through these contaminated areas and may pick up infested soil or litter on their feet and transfer it to new sites. A study was therefore set up to analyse how frequently *Phytophthora* could be isolated from the soil or litter attached to people's boots, particularly those walking in woodlands and gardens known to be infested with *P. kernoviae* and/or *P. ramorum*. The study, which started in July 2004, has shown that in total more than 30 percent of samples collected from walker's boots were contaminated with *Phytophthora*. The most commonly occurring species was *P. citricola*, but 10 to 15 percent of the *Phytophthora* positive samples contained either *P. ramorum* or *P. kernoviae.* The source of inoculum could be fragments of the infected leaves which are shed from infected *Rhododendron* and rapidly break down as they incorporate into the litter layer in affected woodlands. Tests have shown that *P. kernoviae* can survive in both air-exposed and litter-embedded infected leaves for more than a year, although there is a decline in the amount of inoculum that survives, indicated by the success of isolation. In air-exposed leaves isolation success dropped from 60 percent to 15 percent over 12 months, and from 78 percent to 18 percent for litter embedded leaves over the same time.

Key words: *Phytophthora kernoviae*, *P. ramorum*, sudden oak death, dissemination, rhododendron, infected foliage.

Introduction

Two invasive *Phytophthora* pathogens, *Phytophthora kernoviae* and *P. ramorum*, appear to be recent introductions in the U.K. (Brasier and others 2004). Since the first finding of *P. ramorum* in southern England in 2002 (Lane and others 2003) around 580 *P. ramorum* outbreaks in nurseries or plant retail outlets have been confirmed, with a further 160 found at outdoor sites (http://www.defra.gov.uk/planth/pramorum.htm) such as gardens, parks and woodlands. In contrast, *P. kernoviae* has been found at only two nurseries, with around 40 outbreaks in woodlands and gardens

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(http://www.defra.gov.uk/planth/pkernovii2.htm). Many of the *P. ramorum* non-nursery outbreaks are in the south-west of England and, in the case of *P. kernoviae*, almost all the outbreaks are in this region. Here the two species have been found to cause foliar and shoot necrosis of species and cultivars of *Rhododendron*; on some of the most heavily infested rhododendron sites the two pathogens have also infected various tree species (mainly *Fagus sylvatica* and *Quercus* spp.) causing bleeding stem lesions. As both these *Phytophthoras* are aerial pathogens their deciduous sporangia, produced on foliage of infected rhododendron, can be dispersed in mists and rain splash on a local basis. However, patterns of disease spread suggest that vertebrate vectors may also aid the dissemination of these pathogens over longer distances. Infected rhododendron leaves are shed and incorporated into the dense litter layer and the inoculum they contain could potentially persist for months, if not years, in the litter layer. People and animals frequently walk through these contaminated areas and may pick up infested soil or litter on their feet and transfer it to new sites. A study was therefore set up to assess (1) how frequently *Phytophthora* could be isolated from the soil or litter attached to people's boots, particularly those walking in woodlands and gardens known to be infested with *P. kernoviae* and/or *P. ramorum;* and (2) how long foliage naturally infected with *P. kernoviae* remained a source of viable pathogen inoculum.

Materials and Methods

Most experimental work was undertaken in the *Phytophthora kernoviae* Management Zone (PkMZ), an area of about 12.95 square km (5.5 square miles) in Cornwall in the south west of England (Anonymous 2004).

Soil and litter from boots

To assess the potential for movement by people, surveyors and scientists working mainly in the PkMZ scraped any leaf litter and soil from their boots just before leaving *P. kernoviae* and *P. ramorum* infested areas. The samples were put into zip-lock bags and sent back to the laboratory for analysis. To determine which pathogen (if any) each sample contained, it was inserted into a fresh green apple to 'bait' for any *Phytophthora*. The apples were incubated at 18 to 20^{o}C and isolations made from any lesions that developed using a modification of Synthetic Mucor Agar which is a *Phytophthora* selective agar medium (Brasier and Kirk 2004). Typically *Phytophthora* lesions on the apples were firm and often visible within 4 to 5 days of inoculation.

Survival in infected foliage

To assess the survival of *P. kernoviae* in naturally infected rhododendron leaves, symptomatic leaves were collected in September 2005 and placed in net bags. Replicated series of these bags were then either placed in the litter layer or suspended 15 to 20 cm above the litter layer in the air; five bags of each were removed at set intervals over a 12 month period. To check for survival of *P. kernoviae*, the leaves were removed from each bag, and then each individual leaf was subject to both direct isolation and water baiting to detect any *Phytophthora* present.

Results

Dissemination via people

The study to assess dissemination of *Phytophthora* by people started in July 2004 and ran over 3 years with about. 400 samples collected from walkers' boots. Overall, more than 30 percent of samples contained *Phytophthora* and within the subset of positive samples the most commonly occurring species was *P. citricola*, but 10 to 15 percent of the samples contained either *P. ramorum* or *P. kernoviae.* Several other aerial and root infecting *Phytophthora* species were also found. These included *P. ilicis, P. citricola* and *P. cambivora*.

Over the year, a seasonal pattern emerged of the time when *Phytophthoras* were most likely to be isolated from the boots of walkers. Positive samples were most frequent in June to July and then again in October to November. The greatest number of negative samples occurred over the summer during August to September. There also appeared to be an increased likelihood of *Phytophthora* occurring in the 'boot' samples if they contained fragments of leaf litter and foliage.

Survival in infected foliage

In 2005, surveys of *Rhododendron ponticum* in the PkMZ *Phytophthora kernoviae* Management Zone identified thousands of infected plants, many with visibly affected foliage which was frequently shed prematurely. When individual rhododendron leaves were selected with a range of symptoms indicative of *P. kernoviae*, around 60 percent of the isolations yielded *P. kernoviae*. When these leaves became part of the litter layer, the frequency of *P. kernoviae* isolation increased even further. After three months, positive isolations for litter-embedded and air-suspended leaf samples were 78 percent and 54 percent respectively. However, after 6 months positive isolations had decreased to 20 percent and 11 percent (litter compared with air); while after a year the percent survival had fallen to 18 percent and 15 percent (litter compared with air). Continuing the experiment beyond this time proved impossible because the litter embedded leaves had become so fragmented they could not be retrieved intact from the bags, although the fragments readily formed part of the soil layer which adhered to boots of people working in the Management Zone.

Conclusions

Overall, these results indicate that human vectors could provide a significant pathway for the spread of quarantine pathogens such as *P. ramorum* and *P. kernoviae* as well as for other *Phytophthora* species. The basis of the inoculum probably consists of fragments of infected leaf litter, and certainly *P. kernoviae* survives in this type of material over many months, presumably as oospores. However, it has yet to be established if *Phytophthora* inoculum carried on boots can initiate a new infection focus in an area remote from the source of *Phytophthora* inoculum, or indeed how often this is likely to happen. In addition, the ability of *P. kernoviae* to persist for many months in air-dried leaves or comminuted leaves in the litter layer suggests eradicating this pathogen from infected sites is likely to be a long term process.

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Spread and Development of *Phytophthora ramorum* in a California Christmas Tree Farm[1]

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Abstract

The risk of conifers being infected by *Phytophthora ramorum* under natural conditions is poorly understood. In California, infected conifers commonly occur as understory plants beneath or adjacent to heavily infected plants like California bay laurel (*Umbellularia californica*). During wet periods, *P. ramorum* is known to produce a copious amount of spores from spots on infected leaves of this epidemiologically-important host. In Oregon, infection on California bay laurel is limited and infection of Douglas-fir (*Pseudotsuga menziesii*) trees has been limited to a few seedlings directly beneath infected tanoak (*Lithocarpus densiflorus*) trees in the regulated area in Curry County.

A number of studies are currently underway that are examining the influence of various environmental conditions, inoculum levels and host phenology on the infection of a number of hosts by *P. ramorum*. Most of these studies are being conducted on hosts growing within various types of mixed forest communities. Currently it is unclear what the level of risk is for infection of conifers in Christmas tree plantations, conifer nurseries, and coniferous forests. Laboratory studies indicated that infection of Douglas-fir seedlings is limited to a brief period of time right after bud break and is dependent on the inoculum concentration they are exposed to. It is unclear if conifer-to-conifer spread of this disease is possible.

To assess the potential risk associated with the movement of *P. ramorum* via infected Christmas trees, a better understanding of the factors that influence infection under field conditions is needed. The spread and development of *P. ramorum* has been monitored since 2005 in a 9.31 ha (23 ac) U-cut Christmas tree farm near Los Gatos, California. Located within a regulated county, this site provides a unique opportunity to study the spread of *P. ramorum* from the interface of a mixed forest containing highly susceptible hosts, into a Christmas tree plantation.

Conifers being grown at this site include Douglas-fir, grand fir (*Abies grandis*), giant sequoia (*Sequoiadendron giganteum*), Scots pine (*Pinus sylvestris*), white fir (*A. concolor*), and California red fir (*A. magnifica*). Some known *P. ramorum* hosts in the infested forest adjacent to the edge of the Christmas tree farm include: California bay laurel, madrone (*Arbutus menziesii*), big leaf maple (*Acer macrophyllum*), toyon (*Heteromeles arbutifolia*), coast redwood (*Sequoia sempervirens*), and tanoak. After mapping the perimeter of the farm to identify areas where ramorum blight was evident, 500 trees in the largest area with a past history of infection were mapped, tagged and measured for height.

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A series of six transects were established in 2005 from the edge of the forest into the Christmas trees in this area to monitor the spread of *P. ramorum*. The length of these transects ranged from 17 to 27 m. In addition to the established Christmas trees, in 2006 container-grown Douglas-fir and grand fir seedlings that had just broken bud, and small rhododendron plants, were also placed along three of these transects at approximately 0, 3.5, 8, and 13 m from the forest edge. The "0" meter locations on the transects were fully beneath overhanging bay branches. In some cases, there were also a limited number of overhanging bay branches above the 3.5 m locations. The level of infection and extent of shoot dieback was assessed on the tagged trees and containerized seedlings periodically during the spring and summer.

In both 2005 and 2006, new shoot infections on the Christmas trees developed only in the spring and initial dieback symptoms were limited to newly expanded shoot tips. Environmental conditions during spring 2005 were much more favorable to initial shoot tip infections than in 2006. In particular, along the six transects where grand fir were underneath the canopy of infected California bay laurel, virtually all of the new shoots were infected shortly after bud break in 2005. The progression of dieback on infected shoots of Douglas-fir and grand fir in 2005 progressed for about 4 weeks after the initial appearance of symptoms, typically spreading about 5 cm into the previous year's growth. The extent of dieback did not increase between early summer and mid-November.

Infection rates and disease severity were also much higher among container grown seedlings that were placed beneath the bay canopy along the interface of the forest and Christmas tree farm in 2005. On May 19, 2005, 81.7 and 94.3 percent of the Douglas-fir and grand fir seedlings, respectively, that had been exposed since April 21, had become infected. The percentage of each seedling that was killed as the result of shoot dieback averaged 52.8 percent for the Douglas-fir and 81.2 percent for the grand fir. In 2006, infection of conifer seedlings and rhododendrons placed along the transects only occurred during exposure periods when precipitation occurred, and when the plants were in close proximity to infected California bay laurel.

Data collected during the past 2 years at this site, indicates that distance from infected plants (predominantly California bay laurel) within the forest is an important factor relating to the infection of the Douglas-fir and grand fir Christmas trees. Most of the infected Christmas trees and seedlings occurred within 4.4 m of the start of the transects. Virtually no infection was evident on Christmas trees that were 5 to 8 m away from the start.

Key words: *Phytophthora ramorum*, *Pseudotsuga menziesii, Abies grandis*, conifers, epidemiology, spread.

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Sporulation of *Phytophthora ramorum* and *P. kernoviae* on Asymptomatic Foliage and Fruit[1]

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Abstract

Phytophthora ramorum and *P. kernoviae* are newly discovered invasive *Phytophthoras* causing leaf necrosis, shoot tip dieback (mostly on ornamental and forest understorey host species) and bleeding cankers on tree trunks of a wide range of plant species. Both pathogens are now present in south-west England. Sporulation occurs on infected shoots and foliage but not on bleeding stem cankers; thus foliar hosts are key in disease epidemiology. During evaluation of a two-year field trial established to assess infection periods and infection incidence, we discovered that naturally infected, asymptomatic leaves supported sporulation of both pathogens. Asymptomatic leaves of 79 percent of rhododendron trap plants exposed to natural inoculum yielded *P. kernoviae* in the first year of field trials and 53 percent in the second year, whereas 36 percent of trap plants yielded *P. ramorum* in the first year and 33 percent in the second. We realized that sporulation occurred because asymptomatic leaves subjected to baiting remained asymptomatic for the duration of the baiting period, but the baits were positive for the pathogen. The foliage of approximately 20 percent of the positive trap plants remained asymptomatic for the duration of the trial and baiting period, indicating that asymptomatic infection can endure for at least 8 days but may be as long as 22 days. In laboratory trials artificially-inoculated leaves and fruits supported *P. ramorum* sporulation, sporangia were consistently observed on asymptomatic leaves and fruit of several Mediterranean species.

Key words: *P. kernoviae, P. ramorum*, asymptomatic, artificially inoculated, foliage, fruit, natural inoculum, trap plants.

Introduction

P. ramorum and *P. kernoviae* are two recently discovered invasive *Phytophthoras* affecting a wide range of ornamental trees and shrubs as well as forest species (Brasier and others 2004). *Phytophthora ramorum* initially associated with shoot tip dieback and foliage necrosis of ornamental plants in nurseries in Europe (Werres and others 2001) is now known to be the cause of high mortality of native oak species and tan oaks (*Lithocarpus densiflorus*) in the coastal forests of California (Rizzo and

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others 2002). *Phytophthora kernoviae* was first discovered in 2003 in the U.K. on bleeding cankers of beech trees (*Fagus sylvatica*) and wild *Rhododendron ponticum* in woodlands and heritage gardens (Brasier and others 2005) but has recently been reported from soil and diseased *Annona cherimoya* (custard apple) fruit in New Zealand (http://www.maf.govt.nz/mafnet/press/240306fungus.htm). The diseases these pathogens cause include leaf necrosis and shoot tip dieback on foliar hosts which comprise mostly ornamental and forest understory species, and bleeding cankers on tree trunks, sometimes resulting in the death of trees.

Both pathogens share commonality in several biological aspects, including the production of deciduous sporangia adapted for aerial dispersal, which is thought to be the main mode of dissemination. Sporangia and zoospores produced from sporangia of *P. ramorum* are considered the primary inoculum propagules responsible for causing epidemics in California (Davidson and others 2005, Rizzo and others 2005). Neither pathogens form sporangia on bole cankers but do produce inoculum on infected foliage and twig cankers of certain host species (Brasier and others 2004, Davidson and others 2005, Rizzo and others 2005). Thus, for both of these *Phytophthoras* foliar hosts are a crucial component of the disease epidemiology because they are the platforms from which epidemics are driven.

Information about the comparative sporulation potential of susceptible hosts as well as prime infection periods and infection intensity under field conditions is essential to assess the risk posed by these pathogens. Thus, field trials were set up in Cornwall, U.K., to investigate this and *in vitro* tests were carried out to assess the comparative sporulation potential of *P. ramorum* on various host species. While processing plant material exposed to natural inoculum it became apparent that sporulation was occurring from asymptomatic foliage. Closer inspection of artificially inoculated material revealed that sporulation occurred consistently on fruit and foliage of certain host species in the absence of any visible symptoms. This paper gives a summarized account of the incidence of sporulation on asymptomatic foliage of plants exposed to field inoculum of *P. kernoviae* and *P. ramorum* during two seasons of observation, and the extent of sporulation on asymptomatic fruit following artificial inoculation with *P. ramorum*.

Materials and Methods

A detailed account of the methods employed in both the *in vivo* and *in vitro* trials are unpublished (Denman unpublished). Here we give a summary of the field trial methods then briefly describe the laboratory methods used in the artificial inoculations.

Field Trials

In the field trials trap plants were used as indicators of pathogen activity. Field study sites were set out at three woodland locations in south-west England, one naturally infected with *P. ramorum*, and the other two naturally infected with *P. kernoviae*. A single infected wild *Rhododendron ponticum* plant approximately 3 m high was selected at each site to serve as an inoculum source plant. Three 2-year-old, potted rhododendron ‘Cunningham’s White’ plants that had been raised in polytunnels were placed in the drip line of the canopy of the inoculum source plant at each site and replaced with new plants every two weeks. The exposed plants were processed in the

field by separating and bagging symptomatic and asymptomatic leaves from each plant. In the laboratory all leaves were surface sterilised by washing in 70 percent ethanol for 30 seconds, rinsing in sterile distilled water for 1 minute and then air-drying. Thereafter isolations were made from the dead-live junctions of symptomatic leaves, and all asymptomatic leaves were baited as described by Denman unpublished). Seven days after asymptomatic leaves were baited the baits were plated on synthetic mucor agar (SMA, Brasier and Kirk 2002) and the leaves in the bait box were examined for symptoms that might have developed during the 7 day incubation period. When this occurred isolations were made from the leaves to determine the cause of the symptoms (Denman and others 2005). If the baits were positive it indicated that sporulation occurred during baiting since the baits were floating approximately 5 to 10 mm above the surface of the leaves, inferring that zoospores swam to the baits and infected them. Data were gathered on whether or not trap plants had symptomatic and asymptomatic leaves on the same plant, the cause of the symptoms on symptomatic leaves, whether asymptomatic leaves were infected with the pathogen, and on the fate of the asymptomatic leaves during baiting as well as whether baits tested positive for the pathogen or not.

In vitro Fruit Inoculation

The artificial inoculation studies carried out with *P. ramorum* are described in detail (Denman unpublished; Moralejo and others 2006). An isolate of the EU1 lineage and one of the NA1 lineage were used to inoculate fruit of *Crataegus monogyna* (Hawthorn), *Laurus nobilis* (European bay tree), *Quercus ilex* (holm oak), *Rosa sempervirens* (rose) and *Smilax aspera* (rough bindweed). A 20 µl drop of 4×10^4 zoospores/ml was placed on the top of each of the fruits. Controls were inoculated with sterile distilled water. Fruit were incubated in moist chambers for 10 days, the first 48 hours in darkness followed by 12 hour/day exposure to fluorescent white light for the remaining 8 days. The percentage of asymptomatic fruit bearing sporangia in each host-isolate combination was recorded 10 days after inoculation. The numbers of asymptomatic fruit bearing sporangia were analysed using a generalised linear model with binomial error and logit link. The predicted proportions from the model were compared using a t-test.

Results and Discussion

Field Trials

Results are presented for two seasons data from June to November 2005 and 2006. There were 11 fortnightly observation frames per year, representing 132 rhododendrons at the *P. kernoviae* sites and 63 at the *P. ramorum* site (which only had 10 observation frames in 2006).

None of the trap plants had 100 percent infected foliage at any of the sites. Most trap plants had both symptomatic and asymptomatic foliage on the same plant but in some cases entire trap plants were visually disease-free at the end of the exposure period (table 1). For plants with both symptomatic and asymptomatic foliage, in the first year of the field trial 79 percent of plants exposed to *P. kernoviae* inoculum yielded the pathogen from asymptomatic foliage, and in the second year the corresponding figure was 53 percent (table 2). For plants exposed to *P. ramorum* inoculum 33 percent or more yielded the pathogen from non-symptomatic foliage in both years.

Table 1—Number of plants showing symptoms after being exposed to natural inoculation events occurring in 14 d exposure periods

Pathogen	*P. kernoviae*		*P. ramorum*	
Year	**2005**	**2006**	**2005**	**2006**
Appearance: Plants completely symptomatic	0	0	0	0
Appearance: Plants completely asymptomatic	$^{3}/_{66}$	$^{15}/_{66}$	$^{1}/_{33}$	$^{7}/_{30}$
Appearance: Plants with both symptomatic and asymptomatic leaves*	$^{63}/_{66}$	$^{51}/_{60}$	$^{32}/_{33}$	$^{23}/_{30}$

*Note: Isolations need to be made to confirm that symptoms are caused by the pathogens (results of this are unpublished).

Table 2—Number of trap plants with asymptomatic leaves that tested positive for *P. kernoviae* or *P. ramorum* after being subjected to a baiting treatment

Pathogen	*P. kernoviae*		*P. ramorum*	
Year	**2005**	**2006**	**2005**	**2006**
Appearance: Plants completely asymptomatic after exposed to potential natural inoculum for 14d	$^{2}/_{3}$	$^{6}/_{15}$	$^{0}/_{1}$	$^{2}/_{7}$
Appearance: Plants with both symptomatic and asymptomatic leaves on the same plant (testing asymptomatic leaves only)	$^{50}/_{63}$	$^{29}/_{51}$	$^{12}/_{32}$	$^{8}/_{23}$

The fate of the asymptomatic leaves that were baited and baits is summarized in *table 3*. For plants that had both symptomatic and asymptomatic leaves on the same plant, 12 to 20 percent of the baited asymptomatic leaves remained asymptomatic for the duration of the baiting period (regardless of pathogen), but gave rise to positive baits. This indicated that sporulation had occurred in the absence of any visible symptoms on leaves. Generally positive baits were obtained from about 60 to 70 percent of plants that had leaves that went on to develop symptoms during baiting process indicating that sporulation also occurred from these leaves (table 3).

More than 50 percent of plants that had completely asymptomatic foliage after exposure in the field to *P. kernoviae* inoculum yielded the pathogen from asymptomatic leaves, while the incidence was lower for those exposed to *P. ramorum* (table 2).

There was a higher incidence of asymptomatic infection and sporulation in year 2 than in year 1. This appears to correlate with weather conditions prevailing in year 2, where there were periods of unusually high temperature and dryness. Additionally, there had been very severe disease and consequent defoliation of the inoculum source plant in year one. With less foliage, inoculum production is likely to have been reduced. Thus the effect of both these factors (inoculum density thresholds and

environment factors, namely, temperature and humidity) on triggering asymptomatic infection and sporulation needs further investigation.

Table 3—Response of asymptomatic leaves and baits to the baiting treatment

			Response		
	Pathogen	**Year**	**Bait only positive**	**Leaves develop symptoms in bait box and baits positive**	**Leaves develop symptoms in bait box, baits negative**
Appearance: Plants completely asymptomatic after exposure to potential natural inoculum	Pk[a]	2005	$^{0}/_{3}$	$^{1}/_{3}$	$^{1}/_{3}$
		2006	$^{0}/_{15}$	$^{6}/_{15}$	$^{0}/_{15}$
	Pr[b]	2005	$^{0}/_{1}$	$^{0}/_{1}$	$^{0}/_{1}$
		2006	$^{2}/_{7}$	$^{0}/_{7}$	$^{0}/_{7}$
Appearance: Plants with both asympomatic and symptomatic leaves on the same plant (testing asymptomatic leaves only)	Pk	2005	$^{10}/_{50}$	$^{35}/_{50}$	$^{5}/_{50}$
		2006	$^{6}/_{29}$	$^{20}/_{29}$	$^{3}/_{29}$
	Pr	2005	$^{2}/_{12}$	$^{6}/_{12}$	$^{4}/_{12}$
		2006	$^{1}/_{8}$	$^{2}/_{8}$	$^{5}/_{8}$

[a] Pk = *Phytophthora kernoviae*
[b] Pr = *P. ramorum*

In vitro fruit inoculation

Sporulation occurred between 3 to 10 days on all fruit types tested. At one end of the scale almost all the rose hips (98 percent) supported sporulation with no indication of lesion development, compared with about 50 percent of hawthorn and European bay berries (table 4). Based on our results we believe that infected fruit may be an important source of inoculum in natural disease systems and could be significant in the nursery trade if fruit is present. Furthermore, in natural environments where disease is present, inoculum transmission through frugivorous birds may be an explanation for the emergence of new long-distance foci in disease outbreaks, the source of which are otherwise difficult to understand. There has been little research carried out on this aspect and more attention is merited.

Although our work has demonstrated that fruit and foliage can be infected and support sporulation without any visual symptoms much more work is required to get a better understanding of the range of conditions and thresholds under which these pathogens exist and behave in this way. Information on the incubation period (the time between infection and symptom development, Shurtleff and Averre 1997) and the latent period (time between infection and that infection producing infectious propagules; M.J. Jeger, Imperial College, personal communication) of both *P. kernoviae* or *P. ramorum* is required. Additionally the effects that inoculum density and environmental conditions, chiefly temperature and humidity, have on disease expression and inoculum production need investigation. Once more is known about the phenomenon of asymptomatic infection and sporulation its significance

with regard to the potential for introduction and spread by these pathogens through plant trade can be evaluated.

Table 4—Sporulation on asymptomatic detached fruit 10 d after artificial inoculation

Host	Common name	No. fruit tested	Fruit asymptomatic and sporulation (%)
Crataegus monogyna	Hawthorn	64	55 c*
Laurus nobilis	European bay tree	80	48 c
Quercus ilex	Holm oak	64	70 b
Rosa sempervirens	Rose	80	98 a
Smilax aspera	Rough bindweed	80	73 b

* Numbers followed by the same letters do not differ significantly (P<0.5).

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The Status of *Phytophthora ramorum* in Ireland[1]

Carmel O'Connor[2] and Elizabeth Gosling[2]

Abstract

This paper reports on the first 2 years of data collected to study the ecology of *Phytophthora ramorum* in Ireland. Since spring 2005, sampling has been carried out for the presence of the pathogen in soil and watercourses from 11 susceptible forest sites in Ireland, using a rapid DNA method in conjunction with morphological identification methods. Each site was sampled twice a year, collecting foliage and using rhododendron leaves as baits for water and soil samples. In June 2005, the pathogen was positively identified as the cause of *Rhododendron ponticum* twig and leaf blight in Killarney National Park, in the southwest of Ireland. The pathogen has since been positively identified at a new site, approximately 32.2 km (20 miles) from Killarney National Park, but has not yet been identified on trees. To date, *P. ramorum* is only found at three sites in the southwest and southeast of Ireland. While it is unknown whether trees will become infected, many potential susceptible hosts of the pathogen are widely distributed among these forests. Work is currently underway to study the susceptibility of Irish flora to the pathogen, as well as the sporulation potential, dispersal, infection, latency and survival of the Irish isolates. This is the first report on the molecular identification of *P. ramorum* in Ireland.

Key words: ITS nuclear DNA marker, PCR, *Phytophthora ramorum.*

Introduction

After the discovery of *Phytophthora ramorum* (Werres and others 2001) in the U.K. in 2002, emergency European Community legislation was implemented to prevent the spread of the pathogen within the European community. Surveys were carried out in nurseries, public and private gardens and susceptible forest sites throughout Ireland by the Department of Agriculture and Food. At nurseries and garden centres the Department of Agriculture and Food inspects the nurseries twice a year and follows all EC legislation when infected material is found. That is, infected material and all susceptible plants within 2 m of the infected plant are destroyed and all susceptible plants within a 10 m radius are retained for at least three months for inspection and testing. From 2003 to 2005, there was a 2 percent reduction in the number of outbreak sites found in nurseries and garden centres in Ireland.

Official surveys carried out since 2003, by the Department of Agriculture and Food, have confirmed the presence of *P. ramorum* in Ireland. This body identifies the pathogen, using only morphological characteristics. The pathogen has only been found at three locations in the wild (fig. 1) and only on *Rhododendron ponticum*.

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Figure 1—Map of 12 sites sampled throughout Ireland during 2005-2006

This is a huge concern as one of the infested sites, Killarney National Park, forms the most extensive semi-natural woodland left in Ireland covering over 1,200 hectares; *R. ponticum* has completely infested upwards of 650 hectares of the park. This park is frequented by thousands of visitors throughout the year, and there are also volunteer groups whose main aim is to clear rhododendron from the park during the summer. The methods of foliage removal and transport are a cause of concern in the spread of the pathogen throughout the park.

One of the aims of our project, which started early in 2004, was to assess the potential of PCR amplification of a molecular marker to identify the pathogen. In addition, the distribution of the pathogen and identification of the possible vectors involved in its movement throughout Ireland would be investigated.

Materials and Methods

Since March 2005, samples of *R. ponticum*, *Ilex aquifolium* (holly) and *Quercus petraea* (sessile oak) have been analysed twice a year from 11 sites, during the months of March to September. Field sampling was directed towards specific sites with known susceptibility characteristics, in other words, the presence of *Q. petraea*

with an understorey of rhododendron. Sampling was carried out primarily by collecting foliage, but also by using water and soil baits, and lateral flow devices, which were supplied by the Central Science Laboratory in York. Baits were made using 25 cm^2 sections of sterile muslin cloth, into which was placed five pieces of sterile gravel, eight pieces of cut up sterile *R. ponticum* leaves, and two pieces of polystyrene packaging to aid flotation near the water surface. The cloth was tied off, not too tight, with 2.5 m of string (Beales 2006).

Isolation of the pathogen was carried out on P_5ARP agar and identification was confirmed using morphological characteristics first, and then conventional PCR (Van Leeuwen and others 2003). DNA was extracted, from homogenised samples, using the Nucleospin plant extraction kit (Macherey-Nagel, Germany). Conventional PCR was carried out using Hughes's primers (Van Leeuwen and others 2003), which amplify a 700 base pair fragment from the ITS region of a nuclear ribosomal RNA gene. The amplified products were resolved by electrophoresis on a 1.5 percent agarose gel for 1 hour at 100 volts. The gel was run in 1XTBE buffer containing 15 µl of ethidium bromide and viewed on a UV transilluminator.

Results

Of the 11 sites we investigated, the pathogen was found at two locations in the southwest of Ireland over the course of two years sampling. These were Killarney National Park and Lauragh, which is located 32.2 km (20 miles) from Killarney National Park (fig. 1).

Our first positive was found in May 2005 on *R. ponticum* foliage in Killarney National Park. Shortly after this, the Forestry Service intensively surveyed the entire boundary of the park and signs were erected throughout the park, forbidding the removal of susceptible foliage. In February 2006, we found the first water positive in Ireland, in Killarney National Park. The site was located just off a main road and consisted of a body of still water which had a stream flowing from it into a large lake. Since there are three interconnected lakes in Killarney National Park, 10 additional water baits were placed along an 8.1 km (5 mile) stretch of the watercourse, with no further positives being found. In total we have found 13 positives in Killarney National Park, from soil, water and *R. ponticum* foliage (table 1).

The second positive site we found was in the village of Lauragh and the pathogen was isolated from five samples of *R. ponticum* foliage only (table 1). This site is a privately owned site and is currently under containment in accordance with the EC legal requirements of findings in the wild.

In October 2003, the Forestry Service located the third site in the Vee, in the southeast of the country (fig. 1). Eradication efforts were carried out under quarantine control measures, but in 2006 the pathogen was still found at extremely low levels. Despite several sampling trips, we have not found a positive in this area using the two methods of detection.

Table 1—Summary of positive sites sampled throughout 2005-2006

Killarney	2006	2006	**Lauragh**	2005	2006
No. samples collected	17	30	No. samples collected	11	18
No. +ve samples	5	8	No. +ve samples	0	5

Conclusions and Future Work

The Department of Agriculture and Food believes that the inoculum level of *P. ramorum* is low in Ireland, being found by both the department and ourselves at a maximum of three sites. This could be the reason why, to date, the pathogen has not been found on trees in Ireland. If efforts to eradicate the pathogen are not increased, it is only a matter of time before an infected tree is found. There is currently no information available to the public on the presence of *P. ramorum* in Ireland.

A GIS model will be developed to determine the epidemiology of the pathogen, which ultimately could be used as a management tool to target threatened forests for early detection, monitoring and protection.

The sporulation potential and infection threshold will also be tested on a range of tree species, focusing primarily on *Q. petraea*, along with *Pseudotsuga menziesii* (Douglas-fir) and *Taxus baccata* (Yew). The Yew forest in Killarney is the most rare habitat type within the Park and the only significant area of Yew woodland left in Ireland. Our investigation will therefore provide important information on the risk posed by the pathogen to native Irish trees. In addition, we will assess the susceptibility of some special flora found in Killarney National Park such as *Trichomans speciosum* (Killarney Fern), probably the rarest plant species in the Park.

Since the first finding of *Phytophthora kernoviae* in the U.K., in 2005 (Brasier and others 2005), the possible presence of the pathogen in Ireland has become a cause for concern. At present, the Department of Agriculture and Food are surveying for the pathogen using morphological characteristics. Therefore, over the next year our sampling regime will also be targeted at *P. kernoviae* using both morphology and real-time PCR as methods of detection.

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Five Years of Monitoring Infection and Mortality in Redwood Tanoak Forests[1]

Richard C. Cobb,[2 3] Shannon C. Lynch,[3] Ross K. Meentemeyer,[4] and David M. Rizzo[3]

Abstract

Rates of disease incidence and tree mortality in redwood-tanoak forests were determined by repeated sampling across a system of 120 plots at five long-term research sites from 2001 through 2006. Plots were located within the known geographic area of *Phytophthora ramorum* in California, ranging from Monterey to Sonoma counties. All overstory species were monitored, but analysis was restricted to three important host species: California bay laurel (*Umbellularia californica*), tanoak (*Lithocarpus densiflorus*), and redwood (*Sequoia sempervirens*). Infection in bay laurel and tanoak varied among years and plots suggesting weather patterns and abundance of susceptible species are important determinants of disease dynamics. Disease incidence was greatest in bay laurel followed by tanoak and redwood. Tanoak stems greater than 10 cm diameter at breast height were killed more frequently than smaller trees. Further analysis will use statistical modeling and validation to evaluate the role of vegetation structure and climate variation on rates of infection and mortality of tanoak. By proving baseline rates of infection and mortality across structurally heterogeneous stands, this effort will help in the design of stand-level management strategies and identification of local areas where control strategies will be most effective.

Key words: Long-term monitoring, infection rates, mortality rates, changes in forest composition.

Overview of Study

Long-term monitoring is necessary to understand the consequences of stand infestation by *Phytophthora ramorum* and the subsequent ecological impacts of sudden oak death. Management efforts require an understanding of infection rates for major overstory species, rates of mortality of susceptible species, and proportional changes in species composition. Management efforts in *P. ramorum* infested forests are currently operating without empirically defined rates of mortality and infection. To develop long-term systematic management plans for California forests we should have a strong modeling framework that allows us to predict the spread and intensification of the pathogen on individual sites, target the most effective control treatments, and evaluate the effectiveness of these treatments. To date, most

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modeling of *P. ramorum* spread has focused on mapping areas at risk and spatial modeling of susceptible-infectious transitions (Hunter and others, this proceedings) however, better understanding at the stand level is needed to evaluate eradication treatments, focus future eradication efforts, and design other stand level management. This study examines stand-level rates and patterns of *P. ramorum* infection in redwood-tanoak forests.

Over the course of five years, 120, 500 m^2 study plots located at five sites were visited annually and monitored for infection and mortality. The study design was somewhat unbalanced with 30 plots at Jack London State Park (Sonoma County) and the Marin Municipal Water District (Marin County), and twenty plots at two sites in Big Sur and two sites in Santa Cruz County (See Maloney and others 2005 for further site information). During the initial survey, all overstory stems greater than 1 cm diameter at breast height (DBH) were measured in each plot. Foliage subsamples (about 3 g) from all trees with tissue symptomatic of *P. ramorum* were removed and returned to the laboratory where *P. ramorum* infection was confirmed by growing colonies on a *Phytophthora* selective media (PARP). High disease incidence was found in bay laurel and tanoak during the initial survey (Maloney and others 2005). In subsequent years, five trees of each overstory species were randomly selected and assessed for infection status. New infections were confirmed by the method described above. Plot level mortality was estimated by randomly selecting five infected trees and assessing their mortality status. Climate factors including precipitation and temperature have important control over levels of *P. ramorum* sporulation (Davidson and others 2005). The influence of climate factors on infection rates were explored with Poisson regression for each overstory species using a categorical descriptor of proportional increase in infection (0 to 5) as the dependent variable and daily precipitation, maximum, and minimum temperature derived from a regional model (Hunter and Meentemeyer 2005) as independent variables. A separate multiple linear regression evaluated cumulative incidence of infection of each overstory species as the dependent variable and tree density of each focus species as independent variables (three species in each model).

Summary of Results

Cumulative proportion of infected trees steadily increased over the course of the five year study period. Of the plots with known infection by *P. ramorum*, disease incidence was greatest in California bay laurel at 90 percent of surveyed trees. Incidence was 80 percent for tanoak and 20 percent for redwood. Disease incidence rate slowed for bay laurel as infection approached 100 percent. Poisson regression analysis revealed positive effects of precipitation in April and May, and negative effects of maximum temperature on temporal patterns of tanoak infection. Year-to-year differences in redwood infection were negatively related to maximum temperature. Plot level estimates of infection incidence of tanoak and redwood were significantly related to density of bay laurel at the plot level.

Cumulative tanoak mortality increased gradually over time. By the end of measurements, proportional tanoak mortality showed exponential increase consistent with the observed logistic pattern of infection. Large diameter tanoak appear to have greater incidence and rate of mortality compared to smaller diameter trees. Within these study plots, tanoak mortality has resulted in an average 40 percent reduction in

tanoak basal area and at current rates, dead tanoak basal area will exceed live basal area in the next five years. Tanoak mortality has resulted in approximately 2.5 percent increases in relative basal area of both redwood and bay laurel. Very little mortality of redwood and bay laurel occurred during the measurement period and observed mortality was not associated with tree or plot infection status suggesting that *P. ramorum* is not a significant cause of mortality in bay and redwood at the five year timescale of this study.

The sites monitored in this study are representative of many infested stands in central California with respect to composition and impacts (See Murphy and others this proceedings). *Phytophthora ramorum* and subsequent impacts of the disease sudden oak death have spread rapidly through these infested forests and resulted in loss of tanoak. Rate of disease spread is alarming and is likely to subsequently impact ecosystem processes that determine rates of nutrient turnover, input of fine and course woody debris, and overstory community composition. Stand level manipulations should be undertaken with recognition that specific recommendations are not possible based on the current state of knowledge and lack of long-term empirical data for forests other than redwood tanoak.

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Influence of Oak Woodland Composition and Structure on Infection by *Phytophthora ramorum*[1]

Nathan Rank,[2] Hall Cushman,[2] Brian Anacker,[2 3] David Rizzo,[4] and Ross Meentemeyer[5]

Abstract

Introduced plant pathogens have major ecological impacts in many parts of the world. While the spread of pathogens can be strongly mediated by the composition and structure of local host plant communities, little is known about effects of plant community structure on invasion dynamics of introduced pathogens. The progress of infection by the invasive pathogen *Phytophthora ramorum* in coastal California woodlands varies greatly among localities, and some of this variation might be explained by local variation in tree species composition and forest structure. The degree to which patterns of *P. ramorum* infection depend on the abundance and type of host species present was examined in 202 randomly located plots within a 275 km^2 region in eastern Sonoma County, California.

The abundance of over- and understory woody species was measured in plots established in 2003. The disease severity of *P. ramorum* in these plots were surveyed in 2004, 2005, and 2006 through timed counts of symptomatic leaves on bay laurel (*Umbellularia californica*), the primary producer of inoculum in mixed oak woodlands. Leaves were collected and cultured from each plot to confirm presence of *P. ramorum*. Presence of canker infections on oak and tanoak hosts were visually assessed. The among-year repeatability of our censuses of symptomatic bay laurel leaves was confirmed through linear regression of square-root transformed values of the number of leaves counted in spring 2005 compared to spring 2006. These analyses revealed that 74 percent of the variation in 2006 symptomatic leaf count was explained by the 2005 leaf counts. The two-year average value is used in all subsequent analyses.

Bay laurel was the most widely distributed woody species, occurring in 97 percent of plots, followed by coast live oak (*Quercus agrifolia*) (72 percent), Douglas-fir (*Pseudotsuga menziesii*) (47 percent), black oak (*Quercus kelloggii*) (45 percent), madrone (*Arbutus menziesii*) (43 percent), Oregon white oak (*Quercus garryana*) (43 percent), and toyon (*Heteromeles arbutifolia*) (41 percent). The considerable variation in vegetation composition across the study area and high replication of plots also allowed for assessment of the influence of host composition on levels of *P. ramorum* infection. Specifically, the assessment determined whether higher levels of symptomatic bay tissue increases the probability of oak

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infection in a plot and if the presence of oak species is associated with lower numbers of symptomatic bay leaves.

The results showed that bay laurel trees were infected more frequently than canker hosts throughout the study area. Symptoms of *P. ramorum* were observed on bay laurel in 89 percent of plots where it was present, with every stem symptomatic in one third of the plots. In contrast, only nine percent of coast live oak stems were symptomatic in the 123 plots where it occurred, and only six percent of black oak stems were symptomatic in the 80 plots where it occurred. In the 37 plots where oak trees with cankers occurred, 80 percent of bay laurel stems exhibited symptoms of *P. ramorum*, whereas only 26 percent of oak stems possessed symptoms. Overall, oak stems with cankers were observed throughout the study area, but most of them were found in the southwestern portion of the study area, the region believed to be first exposed to the pathogen. The probability of symptomatic coast live oak trees in a plot was positively related to the number of co-occurring symptomatic bay laurel stems.

Plots containing coast live oak (n = 74), black oak (n = 38), both oak species (n = 32), and no oak species (n = 36), were compared using an analysis of variance to determine if the presence of oak canker hosts is negatively related to number of symptomatic bay leaves (using only plots where bay laurel was present). Number of symptomatic bay laurel leaves was significantly lower in plots where coast live oak was present than in plots where it was absent. In addition, number of symptomatic bay laurel leaves was negatively related to the number of coast live oak stems. In contrast, presence of black oak was not related to the number of symptomatic bay laurel leaves. Finally, strong positive relationships between the number (or proportion) of bay stems and total (or mean) number of symptomatic bay leaves were observed, and these relationships did not depend on the presence or absence of oak species.

Taken together, these results suggest that *P. ramorum* spreads among bay laurel in advance of infection on canker hosts, which emphasizes the important role this foliar host plays in the establishment of *P. ramorum* in oak woodlands. The relationship between oak density and disease severity in bay laurel may arise from two possible mechanisms. If disease intensification in bay laurel is density dependent, disease should be lower where the density of bay laurel stems is lower, for example in plots with higher numbers of oak stems. On the other hand disease establishment in bay laurel may be lower in oak dominated plots because these sites are generally warmer, drier habitats not as suitable for *P. ramorum* growth and survival. Further research will distinguish the relative importance of these alternatives.

Key words: Tree species composition, Sonoma County, plant community structure.

Landscape Connectivity Influences the Establishment of *Phytophthora ramorum*[1]

T. Emiko Condeso[2] and Ross K. Meentemeyer[3]

Abstract

As the emergence of invasive pathogens and their impacts on ecological communities increases, so has the interest in understanding how landscape pattern (in other words the configuration and composition of suitable habitat) affects their establishment and spread. Plant pathogen invasions are inherently spatial, but few studies have demonstrated the role of landscape pattern on disease dynamics. In this study, we investigated two hypotheses: Does the spatial pattern of host habitat predict *Phytophthora ramorum* disease severity, and is this relationship scale-dependent?

To examine this question, we first mapped the spatial distribution of suitable habitat for *P. ramorum* in a 20.25 km^2 region on Sonoma Mountain (Sonoma County, California, U.S.) using ADAR multi-spectral imagery. We calculated three simple metrics of landscape pattern that described the size, shape, and connectivity of host woodland using Fragstats 3.3; these were woodland area, perimeter/area ratio and patch cohesion. To examine the spatial scale at which *P. ramorum* responds to landscape pattern, each metric was computed using a multi-scale nested approach. The metrics of area, shape and connectivity were calculated for nested landscapes of increasing size surrounding each sample plot. Each nested landscape was delineated by a circular boundary centered on the plot, with increasing radii of 50 m increments from 50 to 500 m. In the field, we established 86 field plots (15 m by 15 m) within habitat patches according to a random-stratified criterion defined by woodland patch size and distance to a forest-grassland boundary. For each plot, we quantified *P. ramorum* disease severity by counting the number of symptomatic leaves on each bay laurel stem greater than 2 cm diameter at breast height (DBH, defined at 1.4 m) for 90 sec. We confirmed the presence of *P. ramorum* in the symptomatic leaves by culturing a sample of leaves from each of plot using standard PARP methods. To control for the influence of local (within-plot) conditions in the analysis, we also measured the following variables within each of the field plots: abundance of bay laurel (*Umbellularia californica*), canopy cover, distance to forest edge and elevation.

The response of cumulative symptomatic leaf count per plot to the pattern of host woodland (area, perimeter/area ratio and patch cohesion) was examined using multiple regression, testing all possible subsets of predictors and identifying the best model using the Akaike Information Citerion (AIC). Standard beta coefficients were used to assess the relative importance of each of the significant predictors in the regression model. Potentially influential plot-level variables were controlled for by including the aforementioned measures of bay laurel abundance, plot canopy cover, distance to forest edge and elevation.

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Phytophthora ramorum disease severity was greatest in plots surrounded by a high proportion of contiguous forest, after accounting for plot-level variables of host abundance, elevation, canopy cover and microclimate. The explanatory power of the model increased with increasing scale up to 200 m. The effect of surrounding woodland area increased from 50 to 200 m scales, with the strongest effect at 200 m. The effect of bay abundance within each plot was fairly consistent across all scales. The effect of canopy cover over plots was highest at small scales, and decreased at larger scales. The effect of plot elevation became significant at the 50 m scale and remained fairly consistent. Mean perimeter-area ratio of host woodland within each nested landscape did not predict leaf count at any scale. Patch cohesion was highly correlated with woodland area at all scales (Pearson's r ranging from 0.77 to 0.92, $p<0.0001$) and was omitted from these models because when evaluated as an alternative variable, woodland area consistently explained more variance in leaf count across all spatial scales.

In summary, both landscape-scale configuration and local composition of host habitat are related to the severity of this destructive forest disease. Determining the spatial scale of a species' response to habitat is critical for understanding movement ranges and dispersal of invasive organisms such as *P. ramorum*. Increased disease severity within contiguous woodlands may have a considerable impact on the composition of such woodlands, with cascading effects on the population dynamics of both host and pathogen.

Key words: Scale, landscape pathology, connectivity, fragmentation.

Influence of Woodland Expansion (1942 to 2000) on the Establishment of *Phytophthora ramorum*[1]

Ross K. Meentemeyer,[2] Nathan E. Rank,[3] Brian L. Anacker,[2 4] David M. Rizzo,[5] and J. Hall Cushman[3]

Abstract

Human land-use practices have resulted in dramatic alterations of forest ecosystems worldwide. By modifying transmission pathways and habitat structure, land use changes are being increasingly implicated in the emergence of infectious plant disease. In this research, we examined the effects of human-related land-cover change on the establishment of the invasive plant pathogen *Phytophthora ramorum*, causal agent of the forest disease sudden oak death. We hypothesized oak woodlands in coastal California have increased in density and expanded into grassland and shrubland areas over the last century due to fire suppression, leading to increased contagion of host vegetation and cooler forest microclimate conditions facilitating disease establishment.

To examine this hypothesis, we assessed forest structure, understory microclimate, and symptoms of infection on *P. ramorum* hosts over two years (2005 and 2006) in 102 15 x 15 m^2 plots within a 275 km^2 heterogeneous forest region of southeastern Sonoma county, California. Within a 150 m radius area around each plot, we mapped types of land cover (oak woodland, chaparral shrubland, grassland, vineyard, and development) in both 1942 and 2000 using detailed aerial photos. During this 58-year period, oak woodland host habitat significantly increased in area, while grassland and chaparral decreased. In addition, mean size of woodland patches substantially increased and the number of woodland patches decreased, leading to a marked decline in spatial heterogeneity of plant communities.

Woodland expansion was a significant predictor of disease severity, expressed as numbers of symptomatic stems and leaves of bay laurel (*Umbellularia californica*), the primary inoculum-producing host in mixed evergreen forests. Path analysis showed that woodland expansion resulted in larger forests with higher densities of the primary host trees (*U. californica*, *Quercus agrifolia*, *Q. kellogii*) and cooler understory temperatures. Together, the positive effects of woodland size and host stem density and the negative effects of understory temperature explained 65 percent of the variation in the number of symptomatic bay laurel trees and 40 percent of the variation in the number of symptomatic leaves per plot. In conclusion, the enlargement of woodlands and closure of canopy gaps facilitated the

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establishment of *P. ramorum* by increasing contagion of hosts and enhancing forest microclimate conditions. Epidemiological studies that incorporate land-use change are rare but may increase understanding of disease dynamics and improve our ability to manage invasive forest pathogens.

Key words: *Phytophthora ramorum*, invasive species, landscape pathology, woodland expansion, land-cover change.

Pathogenicity of *Phytophthora* Species Isolated From Rhizosphere Soil in the Eastern United States[1]

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Abstract

Pathogenicity of seven *Phytophthora* species was assessed by inoculation of stem and foliar tissues of oak species (*Quercus* spp.) native to the eastern United States. *Phytophthora cambivora*, *P. cinnamomi*, *P. citricola*, *P. europaea*, *P. quercina* 'like', *P*. sp1 and *P*. sp2 were inoculated into stems of 1-year-old greenhouse seedlings and 2-year-old oaks grown under field conditions. Stem inoculations were conducted for 2-month periods in June and September (summer inoculation) and in October-December (fall inoculation). Stem inoculation was conducted by inserting an agar plug containing mycelium on a U-shaped wound, made aseptically about 3 cm above the root collar. Oak species used for 1-year-old stem inoculations included *Q. bicolor*, *Q. macrocarpa*, *Q. montana*, *Q. palustris*, and *Q rubra*; 2-year-old seedlings included *Q. alba*, *Q. montana*, *Q. palustris*, *Q. rubra* and *Q. velutina*. Leaf inoculations also were conducted using oak and understory plant species to evaluate the ability of the *Phytophthora* species to infect foliar tissue. These tests included foliage of the six oak species that were tested as part of the stem inoculation experiments and foliage of *Q. alba*, *Q. imbricara*, *Q. robur, Castanea dentata*, *Kalmia latifolia* and *Rhododendron maximum.* Two leaf age categories were used: leaves that were fully developed and up to 3 months in age; and, leaves that were 3 to 6-months old. Wound and non-wounded treatments were applied to each age category.

When 1-year-old seedlings grown under greenhouse conditions were tested, *P. cinnamomi* and *P. citricola* were the most aggressive species producing significantly larger lesions on all seedlings during the two inoculation periods. *Phytophthora quercina*-like and *P*. sp1 did not cause lesions on any seedlings. Significant variation in lesion sizes did not exist for the other *Phytophthora* species that produced lesions. Of the oak species tested, *Q. palustris* was the most resistant oak, although *P. cinnamomi* and *P. citricola* were able to produce significantly larger lesions on this host when compared to the controls. The largest lesions were produced on *Q. rubra* and *Q. montana*. Generally, no significant difference in lesion size was detected when the summer and fall inoculation periods were compared using ANOVA. Exceptions occurred when *Q. macrocarpa* were inoculated with *P. cambivora* and *P. citricola* during the fall resulting in significantly larger lesions and on *Q. rubra* inoculated with *P. cinnamomi* and *P. cambivora,* which the latter produced significantly larger summer lesions when compared to those made in the fall.

When lesions were formed on the 2-year-old field-grown seedlings, generally lesions that resulted from summer inoculations were larger than those made in the fall. Summer inoculations with *P. cambivora*, *P. europaea*, and *P*. sp2, resulted in significantly larger

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lesions when compared to the controls, however, the same differences were not significant when fall inoculations were compared to the controls. For *P. cinnamomi*, the lesions produced by this isolate were significantly reduced but still larger than the control inoculations during either time period. However, on *P. citricola* lesions were significantly larger than the controls and remained unchanged when spring and fall inoculations were compared. *Phytophthora quercina*-like and *P.* sp1 did not cause lesions on any seedlings during either inoculation period. When oaks were ranked for their susceptibility based on lesion formation, *Q. montana* was the most susceptible and *Q. palustris* the least susceptible species, but ANOVA detected no large variations in susceptibility among the oak species.

For the foliar experiments, generally young foliage was more susceptible than mature foliage when lesion sizes were compared. Wounding also resulted in more consistent infections and larger lesions. The most invasive species was *P. citricola*, but *P. cinnamomi, P. cambivora* and *P. europaea* also were able to cause infections. *P. quercina*-like was the only isolate that was unable to produce lesions significantly larger than the controls. The largest lesions were observed on young wounded foliage of *Q. rubra* and *C. dentata*. For mature wounded foliage, lesions developed best on *K. latifolia, Q. alba and C. dentata*. Young and mature wounded foliage of *Q. imbricara, Q. bicolor* and *Q. palustris* were the most resistant to lesion formation with no *Phytophthora* species causing any significant infections. In experiments using mature unwounded foliage, generally none of the *Phytophthora* species were able to cause infections except on *Q. alba*.

These artificial inoculation experiments, utilizing young oak seedlings, demonstrated the pathogenicity of the *Phytophthora* isolates collected from oak forests in the eastern United States. Under greenhouse conditions the *Phytophthora* species gave variable results with regard to the lesions they produced but generally each species performed consistently when summer and fall inoculation periods were compared. However, when 2-year-old field grown seedlings were used, the lesion sizes were significantly reduced in fall experiments suggesting an effect of lower, late season temperatures. The experimentation implies that the susceptibility of an oak species needs to be evaluated over an extended time period so that the environmental components that influence the aggressiveness of these organisms under field conditions can be determined.

Key words: *Phytophthora* species, oaks, pathogenicity.

Phytophthora Species Associated With Stem Cankers on Tanoak in Southwestern Oregon[1]

Paul Reeser,[2] Wendy Sutton,[2] and Everett Hansen[2]

Abstract

In effort to eradicate *Phytophthora ramorum* from Oregon forests, tanoak over its entire range in southwestern Oregon is surveyed intensively for stem disease. Pieces of bark from the leading edge of tanoak stem cankers were plated on cornmeal agar amended with 10 ppm natamycin, 200 ppm Na-ampicillin, and 10 ppm rifamycin SV (CARP) to favor the isolation of *Phytophthora*. *Phytophthora ramorum* was usually identified on the isolation plates based on the presence of characteristic hyphae, chlamydospores, and sporangia. Hyphae from the leading edge of other colonies resembling *Phytophthora* were isolated into pure culture. Isolates were grown out to obtain morphological features and DNA extracts for identification. Selected isolates were tested for pathogenicity by inoculating stems of tanoak seedlings. *Phytophthora* species isolated from tanoak stem cankers include the following: *P. cambivora*, *P. cinnamomi, P.* species "Pg chlamydo," *P. gonapodyides*, *P. nemorosa*, and the proposed new species *P. siskiyouensis*. An additional isolate may represent a new species of *Phytophthora* that has not yet been described.

Key words: *Lithocarpus densiflorus*.

Introduction

Prior to activities associated with sudden oak death eradication efforts in southwestern Oregon little or nothing was known about *Phytophthora* species occurring on tanoak in this area. During the course of *Phytophthora ramorum* surveys other species of *Phytophthora* were obtained from diseased tanoak stems. This presented an opportunity to learn about the *Phytophthora* species associated with tanoak stem cankers and mortality.

Materials and Methods

Diseased tanoak trees were located by aerial or ground survey during *P. ramorum* detection efforts. Observations on crown condition (leafless, brown, fading, green) and canker characteristics (position on the stem, percent girdling, presence of bleed spots) of each diseased tanoak were recorded.

All suspect trees were sampled to confirm *P. ramorum* infection. Samples from the leading edge of stem cankers were plated in corn meal agar amended with 20 ppm Delovcid® (50 percent Na-natamycin), 200 ppm Na-ampicillin, and 10 ppm rifamycin

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SV (CARP). Isolates resembling *Phytophthora* species, but not characteristic of *P. ramorum,* were purified on fresh CARP, then transferred to corn meal agar amended with 20 ppm β-sitosterol (CMAβ). Isolates were stored as mycelial plugs on CMAß in sterile water with and without chopped hemp seed.

Sporangia were produced in natural stream water (filtered to 5 µm) from margins of colonies grown on 15 percent clarified V8 agar amended with 20 ppm β-sitosterol (V8S). Oogonia from homothallic isolates were produced on V8S in single culture. Heterothallic isolates were induced to form oogonia by pairing with standard A1 and A2 testers of *P. cinnamomi* on V8S. Structures for microscopic observation were fixed and preserved in 3.7 percent formaldehyde. Isolates were identified by comparison of features with published descriptions (Brasier and others 2003, Hansen and others 2003, Reeser and others 2006, Waterhouse 1963).

Representative isolates from each species identified were selected for DNA sequencing. DNA extracted from mycelium grown on CMAβ was used as template to amplify nuclear rDNA internal transcribed spacer (ITS) for sequencing. ITS sequences were compared with Genbank database using BLAST Search to aid identification.

Pathogenicity of selected isolates from each species was tested in green twigs of tanoak seedlings by placing mycelium grown on CMAβ over a pinprick wound and covering with Parafilm®. After seven days lesions were measured and photographed. Leading edges of lesions were plated in CARP for re-isolation.

Results and Discussion

During 2006 stem cankers from 263 tanoak trees were sampled. Of these, 97 were culture negative for *Phytophthora* species and 123 were culture positive for *P. ramorum*. Other *Phytophthora* species isolated from tanoak stem cankers were *P. cambivora* (four isolates), *P. cinnamomi* (one isolate), *P. gonapodyides* (four isolates), *P. nemorosa* (31 isolates), an undescribed *Phytophthora* species (one isolate), and the proposed new species, *P. siskiyouensis* (three isolates). *Phytophthora*. taxon "Pg chlamydo" was isolated from a tanoak stem canker in 2005.

Examination of tree and canker attributes suggested that there was no relationship between crown condition, or canker appearance, and *Phytophthora* species isolated. Based on the field observation data, cankers yielding other *Phytophthora* species were indistinguishable from cankers yielding *P. ramorum*. The geographic distribution of the isolates showed no apparent pattern for occurrence, except that all isolates of the proposed new species, *P. siskiyouensis,* were recovered from trees growing in coastal stream drainages.

Selected isolates of *P. cambivora*, *P. cinnamomi*, *P. gonapodyides*, *P. nemorosa*, an undescribed *Phytophthora* species, the proposed new species, *P. siskiyouensis*, and *P.* taxon "Pg chlamydo" were tested for pathogenicity in green tanoak stems. Four isolates of *P. gonapodyides* produced the largest lesions, followed by one isolate of *P. nemorosa* and one isolate of *P. cambivora*. Each pathogen was re-isolated from its respective lesion. The other species tested did not produce noticeable lesions when inoculated into green tanoak stems.

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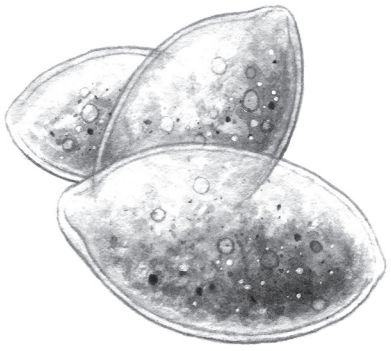
Genetics



Phytophthora ramorum

What Can Availability of the *Phytophthora ramorum* Genome Do for Us?[1]

Niklaus J. Grünwald[2]

Abstract

The complete genomes of *Phytophthora ramorum* and *P. sojae* have recently been sequenced. Of the 19,027 predicted genes in *P. sojae* and 15,743 gene models in *P. ramorum*, 9,768 are predicted to have the same function. These two genomes both revealed a rapid expansion and diversification of many protein families associated with plant infection including different classes of pathogen effectors. Two protein motifs (RxLR and dEER) are shared by the four known effectors in plant pathogenic oomycetes. Genome analyses identified a diverse superfamily of approximately 350 genes in *P. ramorum* that share these motifs. These have been termed avirulence homolog (Avh) genes. We were able to clone homologous Avh loci from the *P. ramorum* sister-taxa *P. lateralis* and *P. hibernalis.* Availability of the *P. ramorum* genome sequence has also resulted in several practical applications including identifying which genes are differentially expressed during different pathogen life-stages and during plant infection. The genome has been mined for molecular loci that can be used for pathogen identification and genotyping. Both the *P. ramorum* and *P. sojae* genomes have been used to select sequence loci for the construction of a phylogeny of the genus *Phytophthora*. The *P. ramorum* genome has also been helpful in finding simple sequence repeats and sequence loci that have been used to study the population structure and migration of clones. Availability of the *P. ramorum* and *P. sojae* genome sequences has already provided advances and a new understanding of *Phytophthora* biology. Many promising new discoveries are sure to follow.

Key words: *Phytophthora ramorum*, comparative genomics, genome.

Introduction

The complete genomes of *Phytophthora ramorum* and *P. sojae* were sequenced in 2004 (Tyler and others 2006). One obvious question arising is what contributions the availability of a genome sequence has for understanding the biology of *Phytophthora* spp. and the implications for management of sudden oak death (caused by *P. ramorum*) in the field. Simultaneous sequencing of two *Phytophthora* genomes allowed for rapid and direct comparison for similarities and differences between these two organisms.

[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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Phytophthora Genomes

To date five Oomycete genomes have or are being sequenced, of which four include Phytophthora species (table 1). *Phytophthora ramorum* and *P. sojae* were sequenced in 2004 at the Joint Genome Institute. The potato late blight pathogen *P. infestans* and the *Arabidopsis* downy mildew pathogen *Hyaloperonospora parasitica* were sequenced in 2006 and *P. capsici* is being sequenced.

Table 1—Background information on Oomycete genomes sequenced to date

Species	Genome size (Mb)	Coverage[1]	Sequencing center[2]
Phytophthora ramorum	65	7.7×	JGI
Phytophthora sojae	95	9×	JGI
Phytophthora infestans	240	8×	BI
Phytophthora capsici	65	2×/20×	JGI
Hyaloperonospora parasitica	75	8×	WUGSC

[1] 2× coverage based on Sanger sequencing and /20× coverage based on 454 sequencing.
[2] JGI= Joint Genome Institute (http://www.jgi.doe.gov/); BI =Broad Institute (http://www.broad.mit.edu/); WUGSC = Washington University, Genome Sequencing Center (accessible via http://phytophthora.vbi.vt.edu/).

Genome Information

Of the 19,027 predicted genes in *P. sojae* and 15,743 gene models in *P. ramorum*, 9,768 are predicted to have the same function. These two genomes both revealed a rapid expansion and diversification of many proteins families associated with plant infection including different classes of pathogen effectors.

Examples of Usage of Genome Data

Pathogen effectors can serve a virulence function on behalf of the pathogen or trigger a rapid defense response in resistant hosts. Two protein motifs (RxLR and dEER) are shared by the four known effectors in plant pathogenic oomycetes. Genome analyses identified a diverse superfamily of approximately 350 genes in *P. ramorum* that share these motifs (Tyler and others 2006). These have been termed a virulence homolog (Avh) genes. We have investigated the molecular evolution of one such group of six Avh genes. Microarray data suggests that four of these genes are expressed in isolate Pr-102 (Press and Grünwald, unpublished). We sequenced the full coding region (approximately 400 bp) and flanking noncoding regions of each gene in the three *P. ramorum* lineages (Goss and Grünwald, unpublished). The number of polymorphic sites within genes ranged from one to 12, suggesting different evolutionary pressures among genes. We have further been able to obtain the sequence of homologous Avh genes in the closely related *P. hibernalis* and *P. lateralis*.

Availability of the *P. ramorum* genome sequence has also resulted in several practical applications. For example, we are investigating which genes are differentially expressed during different life-stages (e.g., mycelium, zoospores) and

during plant infection using microarrays developed from the current genome annotation (Press and Grünwald, unpublished). Figure 1 shows transcripts of

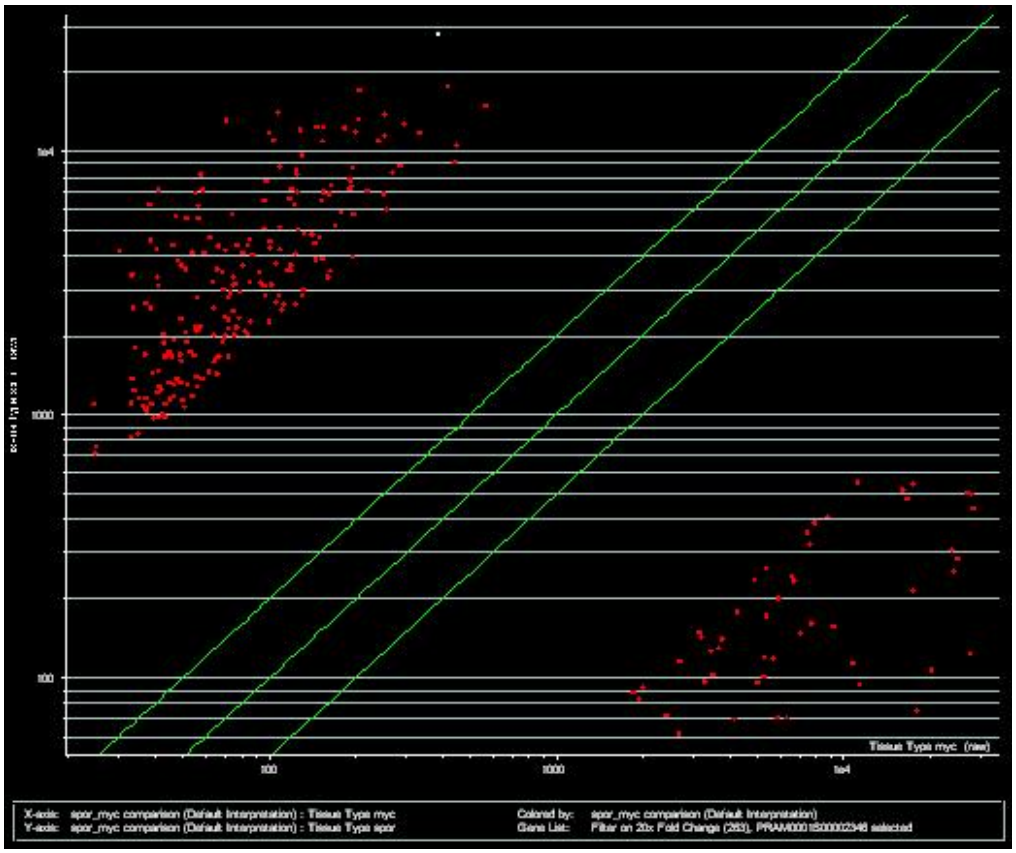

Figure 1—Red dots represent transcripts of *P. ramorum* with greater than 20-fold change in expression in mycelium as compared to sporangia (Press and Grünwald, unpublished) (x-axis: mycelium; y axis: sporangia).

P. ramorum that are more than 20-fold over or under-expressed in mycelial (x axis) versus sporangial (y axis) tissue.

The genome has been mined for molecular loci that can be used for pathogen identification and genotyping. The genomes have been helpful in selecting sequence loci that can be used for constructing a phylogeny of the genus *Phytophthora* (http://www.phytophthoradb.org/). Kang and colleagues have selected eight sequence loci that are phylogenetically informative to develop a multi-gene phylogeny of the genus *Phytophthora* (S. Kang, personal communication). Many studies have developed other molecular markers that can be used for pathogen identification using PCR (Hayden and Rizzo 2004; Kong, and others 2004; Tomlinson and others 2005; Hayden and others 2006; Hughes and others 2006a; Hughes and others 2006b; Ioos,and others 2006; Schena and others 2006; Tooley and others2006) or single nucleotide polymorphisms (Kroon and others 2004; Bilodeau and others 2006).

The *P. ramorum* genome has also been helpful in finding simple sequence repeats and sequence loci that have been used to study the population structure and migration

of clones (Prospero and others 2004, Ivors and others. 2006, Prospero and others in press). These studies have identified three distinct clonal lineages of *P. ramorum* in the U.S. that currently show no evidence of sexual recombination although both mating types have been found to co-occur in a few locations.

Outlook

Availability of the genome sequences has already provided advances and a new understanding of *Phytophthora* biology. Importantly, genome availability has provided timely tools for pathogen identification and genotyping. Availability of *Phytophthora* genomes has also provided leverage for obtaining additional resources for *Phytophthora* research. The Oomycete research community has been energized and a flurry of studies have already resulted from the availability of the genome sequences. Some of this research was recently highlighted in a special issue of Molecular Plant Microbe Interactions (Govers and Gijzen 2006, Jiang and others 2006, Krampis and others 2006, Lamour and others 2006, Meijer and Govers 2006, Meijer and others 2006; Tripathy and Tyler 2006, Zhang and others 2006). Many promising new discoveries are sure to follow.

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Population Structure of *Phytophthora ramorum* in Oregon [1]

Simone Prospero,[2] Jennifer Britt,[3] Niklaus Grünwald,[4] and Everett Hansen[5]

Abstract

Phytophthora ramorum is infecting plants in Oregon forests and nurseries. In this study, we analyzed the population structure of the *P. ramorum* in Oregon from 2001 to 2004 using microsatellites. The *P. ramorum* population in Oregon is characterized by low genetic diversity, significant genetic differences between nursery and forest populations, and no evidence of sexual recombination. The forest population is dominated by a single multilocus genotype in all four years, indicating the persistence of pathogen inoculum. In nurseries, identical genotypes were not recovered during both sampling years (2003 and 2004), suggesting pathogen eradication was effective. This research highlights the continued importance of sanitation and quarantine in nursery production to prevent further introduction and spread of *P. ramorum*.

Key words: *Phytophthora ramorum*, population genetics, microsatellites.

Introduction

P. ramorum was first detected in Oregon forests in 2001 and nurseries in 2003. Quarantine and eradication efforts are currently in operation. Nevertheless, newly infected plants are detected every year. In order to determine the persistence and spread of *P. ramorum* in Oregon forests and nurseries, we designed a study using population genetic techniques. We investigated the following questions: (1) what is the population structure of *P. ramorum* in Oregon, (2) is sexual recombination taking place, (3) and are the eradication and quarantine efforts working?

Materials and Methods

A total of 323 isolates of *P. ramorum* were collected from 2001 to 2004; 272 were recovered from the infested forest and 51 were obtained from nurseries. About 60 percent of the forest isolates were collected from infected trees or shrubs, thirty isolates were obtained from baits in seven streams and 29 isolates were obtained from soil samples. The 51 nursery isolates originated from infected plants grown in 15 nurseries.

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The 2001 to 2004 isolates of *P. ramorum* were screened for alleles at 10 microsatellite loci. The di-nucleotide and tri-nucleotide loci were previously isolated from an enriched genomic library as described by Prospero and others (2004). Four additional loci were identified by screening the genome of *P. ramorum* (available online at: http://genome.jgi-psf.org/) for common tetra-nucleotide repeats.

Results and Discussion

This study suggests that the nursery and forest populations of *P. ramorum* in Oregon are distinct. A total of 33 multilocus genotypes were identified among isolates collected from 2001 to 2004; 22 genotypes were only recovered from the infested forest, nine genotypes were only detected in nurseries, and two genotypes were observed both in the infested forest and in nurseries. Genetic variation within populations, estimated with Stoddart and Taylor's G index, was very similar for forest populations of *P. ramorum* from all four sampling years. Support for similarities in the *G* values among populations was tested with bootstrapping at a confidence level of 90 percent. Including the nursery isolates in the 2003 and 2004 populations of *P. ramorum* caused an increase in the genetic variation both in 2003 and 2004. Genetic variation among populations was estimated with Weir & Cockerham's (1984) coefficient of differentiation θ and distance trees based on Nei's (1972) genetic distance. No differences were found among the four annual forest populations ($\theta = 0.0006$, $P = 0.302$). The forest and nursery populations were significantly different ($\theta = 0.316$, $P < 0.001$) as were the four annual *P. ramorum* populations when forest and nursery isolates were combined ($\theta = 0.0283$, $P < 0.001$). The nursery populations in 2003 and in 2004 were also significantly different ($\theta = 0.160$, $P < 0.001$). The trees based on Nei's genetic distance showed that the four annual forest populations of *P. ramorum* were genetically very close (0.003, fig. 1A). A considerably larger genetic distance was observed between the forest population and the nursery population (0.235, fig. 1C). Comparative trees derived from clone-corrected data sets showed qualitatively similar results (0.015 and 0.160 fig. 1B and D respectively).

Several results indicate clonal reproduction in the *P. ramorum* population in Oregon. First, there is low genotypic diversity, with most isolates belonging to a dominant genotype. Second, the observed heterozygosity is higher than that expected under Hardy-Weinberg equilibrium, resulting in a negative inbreeding coefficient (F_{IS}). In diploid organisms, extreme clonality often results in considerable heterozygote excesses (Balloux and others 2003). Third, the index of association indicated the presence of significant linkage disequilibrium in all populations when a single representative of the European genotype was added to the data set (Dobrowolski and others 2003).

Our study reveals that *P. ramorum* eradication efforts in nurseries are working. The nursery population of *P. ramorum* was not dominated by a single multilocus genotype. The most common genotype comprised 25.5 percent of the isolates and was found in six out of 15 nurseries. Each of the other 10 genotypes had a frequency of 2 to 20 percent. None of the 11 nursery genotypes found in 2003 and 2004 was detected in both sampling years. This suggests effective eradication of nursery infestations in 2003, followed by new introductions from different sources in 2004.

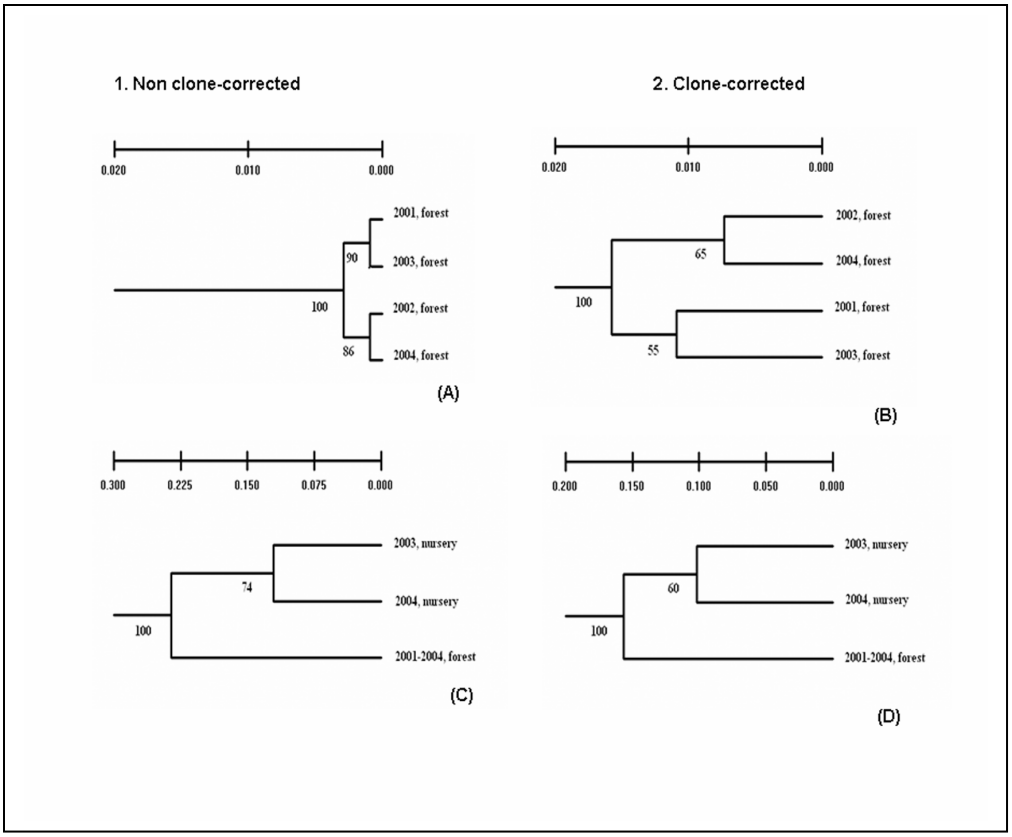

Figure 1—Phenogram constructed using the unweighted pair-group method of averages (UPGMA) algorithm based on the Nei's (1972) genetic distance of populations. Statistical support for branches was obtained using 1000 bootstrapped samples of the data set. The numbered bars show the distance. In A and B, only forest isolates are included in the analysis. In C and D, forest and nursery isolates were included in the analysis.

Our study also suggests that quarantine efforts in the Oregon forest are highly effective at stopping the introduction of new infections to the forest. In all four sampling years, the forest population was dominated by a single multilocus genotype which persisted over all years and was found in all main infection centers. All but two other genotypes occurred at less than 5 percent frequency. Four genotypes were found in all four sampling years, whereas 12 genotypes were only detected in one particular year. This is suggestive of a likely single introduction with the persistence of an inoculum source responsible for new infections as it spreads through the forest. Destruction of all potential hosts growing around a symptomatic plant clearly reduces the inoculum levels and the spread of the pathogen, but has not completely prevented continuing infections.

The presence of different *P. ramorum* genotypes in Oregon forests and nurseries emphasizes the importance of prevention and sanitation practices in nurseries in order to reduce the risk of introducing new populations of *P. ramorum* into forest ecosystems.

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Mitochondrial Genomics in the Genus *Phytophthora* With a Focus on *Phytophthora ramorum*[1]

Frank N. Martin[2] and Paul Richardson[3]

Abstract

The mitochondrial genomes of *Phytophthora infestans*, *P. ramorum* and *P. sojae* have been sequenced and comparative genomics has provided an opportunity to examine the processes involved with genome evolution in the genus *Phytophthora*. This approach can also be useful in assessing intraspecific genome evolution and identification of cytoplasmic markers that will be useful in population studies. Polymorphisms have been observed in the mitochondrial genomic sequences of *P. ramorum* isolates from North America and Europe (13 single base changes and an insertion of 180 bp in the European isolate). The development of seven primer pairs for amplification and sequencing of some of these polymorphic regions has identified four mitochondrial haplotypes. The two most common ones represent the North American and European lineages, but there also is a third haplotype representing the third lineage of the pathogen (from Washington State) and a fourth haplotype found in several isolates recovered from the forest ecosystem in Oregon.

Key words: Intraspecific polymorphisms, mitochondrial molecular markers.

Introduction

Due to their rates of sequence divergence, mitochondrial genomes can provide a valuable tool for studying evolutionary relationships among groups of organisms as well as provide molecular tools for isolate identification to a species, and sometimes subspecies level. The mitochondrial genomes in the genus *Phytophthora* are uniparentially inherited (from the maternal parent), in the size range of 40 kb and map to a circular orientation (reviewed in Martin and others 2007). Unlike the closely related genus *Pythium*, the mitochondrial genomes of *Phytophthora* do not have a large inverted repeat (IR; in *Pythium* the IR can represent approximately 75 percent of the genome, McNabb and others 1987). However, based on restriction mapping and Southern analysis, a short inverted repeat of 0.5 to 0.9 kb in size was observed in *P. megasperma* (Schumard-Hudspeth and Hudspeth 1990). The IR is believed to stabilize the mitochondrial genome and reduce the potential for genome rearrangements.

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The mitochondrial genomic sequences for the four mitochondrial haplotypes of *P. infestans* have been published (Paquin and others 1997, Avila-Adame and others 2006) and comparative genomics revealed that intraspecific polymorphisms were due to both single nucleotide substitutions dispersed throughout the genome as well as length variations caused by insertions/deletions that occurred primarily in two locations (Avila-Adame and others 2006). More recently, the mitochondrial genomic sequences of *P. ramorum* (California forest isolate) and *P. sojae* have become available (Martin and others 2007). These species had the same 68 coding regions that were identified in *P. infestans*, inferred to encode mitochondrial respiratory chain proteins, subunits of the mitoribosome, ribosomal RNAs, tRNAs, and unassigned ORFs that were conserved among the *Phytophthora* spp. Comparative genomics among these species indicated the gene order for *P. ramorum* and *P. sojae* were identical, but comparison with *P. infestans* revealed two inversions. It was also apparent that certain regions of the genome were more polymorphic than others and the terminal ends of the inversions in *P. infestans* corresponded to regions of greater polymorphisms in comparisons between *P. ramorum* and *P. sojae*. Furthermore, unlike *P. infestans* and *P. sojae,* there is a small inverted repeat (1,150 bp) present in *P. ramorum* that encodes a unique open reading frame and a duplicate copy of *trnR(ucu)*.

An intraspecific polymorphism in the mitochondrially encoded cytochrome oxidase 1 gene has been identified that differentiates North American from European isolates of *P. ramorum* (Kroon and others 2004), but it is possible that genome wide comparisons between these regional populations would identify further differences that could be used to classify additional mitochondrial haplotypes in this species. The objective of this project was to evaluate intraspecific variation in the mitochondrial DNA of *P. ramorum* using a whole genome approach and to design additional molecular markers that can be used to identify a greater number of haplotypes. The availability of these markers would be useful in population studies of the pathogen by providing a cytoplasmic marker that would compliment the results from nuclear markers.

Materials and Methods

To evaluate intraspecific variation in mitochondrial genomic sequences for *P. ramorum* (CBS 101553, a European isolate) the mitochondrial DNA was purified from total DNA using CsCl + bisbenzimide density ultracentrifugation (Martin and Kistler 1990) and sent to the production facilities at the Joint Genomic Institute where standard techniques were used to construct and sequence a clone library (detailed protocols are available at http://www.jgi.doe.gov/sequencing). The sequences were aligned and contigs were assembled at the JGI sequencing production facility with final verification of the consensus sequence done with Sequencher ver. 4.6 (Gene Codes, Ann Arbor, MI). Annotation of coding regions and prediction of ORFs was done with DS Gene v1.5 (Accelrys, San Diego, CA) using the universal genetic code. Identification of protein- and rRNA-encoding genes was done by comparison with sequences reported for *P. ramorum* (Martin and others 2007; DQ 832718) and BLAST analysis to other sequences in GenBank. Genes for tRNAs were confirmed using tRNAscan SE v1.1 (Lowe and Eddy 1997; http://www.genetics.wustl.edu/eddy/tRNAscan-SE/). Pairwise comparisons among

genomes were made using mVISTA (http://genome.lbl.gov/vista/servers.shtml) as described previously (Martin and others 2007).

Primers spanning the polymorphic loci were designed and the regions amplified for a total of 39 isolates representing a range of cultures recovered from nurseries and forests in California, Oregon, and Washington State, as well as European countries. Amplicons were sequenced and Sequencher or DS Gene was used to analyze the alignments.

Results and Discussion

The mitochondrial genome of the European isolate of *P. ramorum* (CBS101553) was identical to the California isolate (DQ 832718) with the exception that there were single nucleotide polymorphisms at 13 positions dispersed throughout the genome and a 180 bp insertion in the European isolate. This level of intraspecific variation is similar to what was observed in comparisons of mitochondrial haplotypes Ia and Ib for *P. infestans* (Avila-Adame and others 2006).

Primers were designed to amplify seven of these polymorphic regions (table 1). Sequence alignments of these amplicons revealed that three primer pairs identified the mitochondrial haplotype found in North America (NA) or Europe (EU).

Table 1—Molecular markers used to determine mitochondrial haplotype in *Phytophthora ramorum*

Marker	Length	# Variable bases	MtDNA Haplotype
1	540 bp	1	I – EU II - NA
2	870 bp	2	I – EU II - NA
3	1,025 bp	5	I – EU II - NA
4	370 bp	2	I – EU II – NA III – Washington State
5	347 bp	2	I – EU II – NA III – Washington State
6	740 bp	2	I – EU II – NA III – Washington State
7	865 bp	4	I – EU II – NA III – Washington State IV – Oregon forest

However, three additional primer pairs also differentiated a third haplotype representing the third lineage of the pathogen that was recovered from Washington State (Ivors and others 2006). Lastly, the seventh primer pair was able to differentiate these three mitochondrial haplotypes as well as a fourth haplotype that was present in several isolates recovered from the forest in Oregon (DNA samples provided by Nik Grünwald, United States Department of Agriculture-Agricultural Research Service (USDA ARS). What this additional haplotype represents is uncertain at this time as more samples need to be examined and compared to nuclear genotypic data.

The ability to identify mitochondrial haplotypes will provide additional markers that can be useful for monitoring specific lineages of the pathogen and when used in conjunction with nuclear markers, will provide a maternally inherited cytoplasmic marker for population genetic studies.

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Modeling

Trientalis latifolia

Predicting Movement of Nursery Hosts Using a Linear Network Model[1]

Steve McKelvey,[2] Frank Koch,[3] and Bill Smith[4]

Abstract

There is widespread concern among scientists and land managers that *Phytophthora ramorum* may be accidentally introduced into oak-dominated eastern U.S. forests through the transfer of the pathogen from infected nursery plants to susceptible understory forest species (for example, *Rhododendron* spp.) at the forest-urban interface. Inspection programs can be made more efficient by identifying locations throughout the U.S. that are most likely to receive infected nursery stock. We develop a spatial network model framework utilizing potential interstate nursery stock movements on a bipartite network, adopting a Bayesian approach to model probabilities of transmission of *P. ramorum* from entry to destination nodes within the network. As the goal of this paper is to present a general model framework, no specific risk analysis is presented. In the face of future discoveries of *P. ramorum* infections in the eastern U.S., instances of models from this framework can be used to help identify sites with a high risk of infection based on observed infection patterns.

Key words: Bayesian analysis, *Phytophthora ramorum*, nursery shipments, transportation network.

Introduction

Although *Phytophthora ramorum* has only been detected in natural forest landscapes in California and Oregon, there is some concern that the pathogen may be accidentally introduced into oak-dominated eastern U.S. forests. A noteworthy potential pathway for such introduction is transfer of the pathogen from infected nursery plants to susceptible understory forest species (for example, *Rhododendron* spp.) at the forest-urban interface. Genetic evidence suggests the commercial plant trade has played a major role in spreading the pathogen (Ivors and others 2004, Ivors and others 2006); indeed, there appears to be continued exchange of *P. ramorum* genetic material between Europe and North America (Rizzo and others 2005). Of more immediate concern, confirmed sporulating hosts include a number of genera, particularly from the Ericaceae family, that are both widely distributed in natural forests of the U.S. and commonly sold as ornamental plants by nurseries (Englander and Tooley 2003, Tooley and others 2004, Rizzo and others 2002, Rizzo and others 2005). Despite current federal restrictions (see APHIS 2007), the interstate shipment of west coast nursery stock remains a serious risk, as is evident from the detection of infected plants in nurseries in Indiana, Maine, and other states in 2006. The United

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States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) and the USDA-Forest Service (USDA-FS) have ongoing national *P. ramorum* survey programs; these programs can be made more efficient by identifying locations throughout the U.S. that are most likely to receive infected nursery stock.

Towards this end, we have been developing a model framework based on a bipartite network incorporating interstate nursery stock movement. The network depicts nursery stock shipment from sources on the west coast to destinations further east where *P. ramorum* has not been detected in the natural forest landscape. We constructed the network using geographic information system (GIS) data layers and relational databases from the U.S. Department of Transportation and other federal agencies. The foundation of our network is the Freight Analysis Framework (FAF_2) Commodity Origin-Destination database (U.S. Department of Transportation 2006). The Commodity Origin-Destination database is a relational database describing the movement of commodities, in both tonnage and monetary value, between statistical regions. The statistical regions in the database consist of major metro areas, the remaining areas of states, and entire states. These regions are treated as nodes in the model network.

In our model framework, probabilities of transmission of *P. ramorum* from source to destination nodes within the network are based on nursery stock flow volumes along the links connecting each origin/destination pair. See equation (2). The 43 data categories in the FAF_2 are not specific enough to break out nursery stock as a specific category of commodity. The nursery stock tonnages are approximated by estimating what proportion of all commodities consists of nursery stock and applying this proportion to each link in our network (US Departments of Commerce and Transportation 2005).

Other approaches to identifying possible paths of *P. ramorum* infection are certainly possible. The model framework in this paper is quite general and requires only the identification of nodes that are sources of infection, nodes that are at risk of becoming newly infected and some measure of the likelihood that *P. ramorum* will be spread from each currently infected node to the currently uninfected nodes. The network we develop using FAF_2 information provides a solid test network for our model and is a good representation of the kind of network and corresponding infection likelihood information that a user of this model would need to construct.

As new infections are observed a Bayesian update of conditional probabilities allows us to make comparative statements about the likelihoods of various paths of infection, identifying likely proximate sources of the infection. The same analysis can identify new sites that are at high risk of infestation through importation of *P. ramorum* from these proximate sources even if *P. ramorum* has not yet been detected. This allows managers to selectively target resources in their efforts to mitigate the spread of *P. ramorum.*

The need for such a tracking mechanism is plain after considering the two maps shown in figures 1 and 2. These figures have the FAF_2 statistical regions as a backdrop. The lines and points in figures 1 and 2 are derived from USDA-APHIS trace forward data (APHIS, unpublished data).

Figure 1 shows the links to regions where *P. ramorum* host plants, not necessarily infected by *P. ramorum*, were shipped by commercial nurseries from California and Oregon during the years 2003 through 2006. These flows are derived from USDOT data (U.S. Department of Transportation 2006). The dots on the figure show the actual location of nurseries that received host plants from California and Oregon. The network of actual shipments resolved to localities rather than regions would be far more complex.

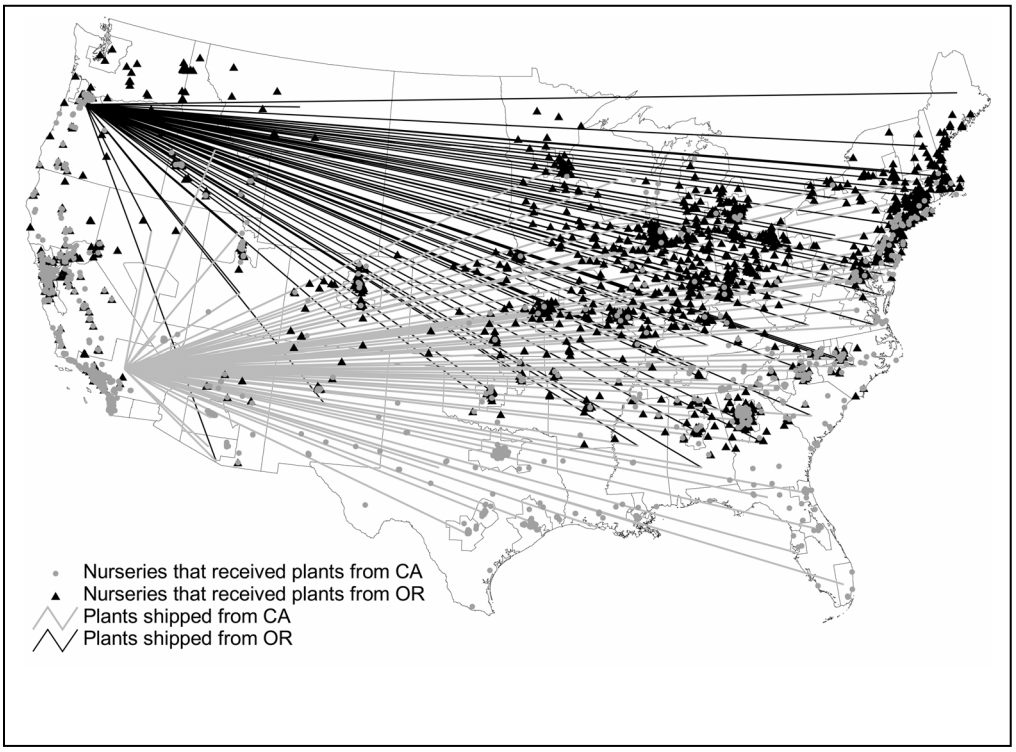

Figure 1—U.S. *P. ramorum* host plant shipping pattern.

Figure 2 shows the dynamic nature of the flow of nursery stock plants that were found to be positive for *P. ramorum*. In this figure we clearly see how the source of infected plants has shifted northward. During the earlier years of 2003 and 2004 southern California was the most common source of detected infected host plants in nursery stock. In 2005 and 2006 northern Oregon became a significant source of infected nursery stock.

The models we propose will help inspectors quickly identify future shifts in the source of *P. ramorum* infected nursery stock, increasing the likelihood of detecting and mitigating the spread of the pathogen past the urban/forest interface.

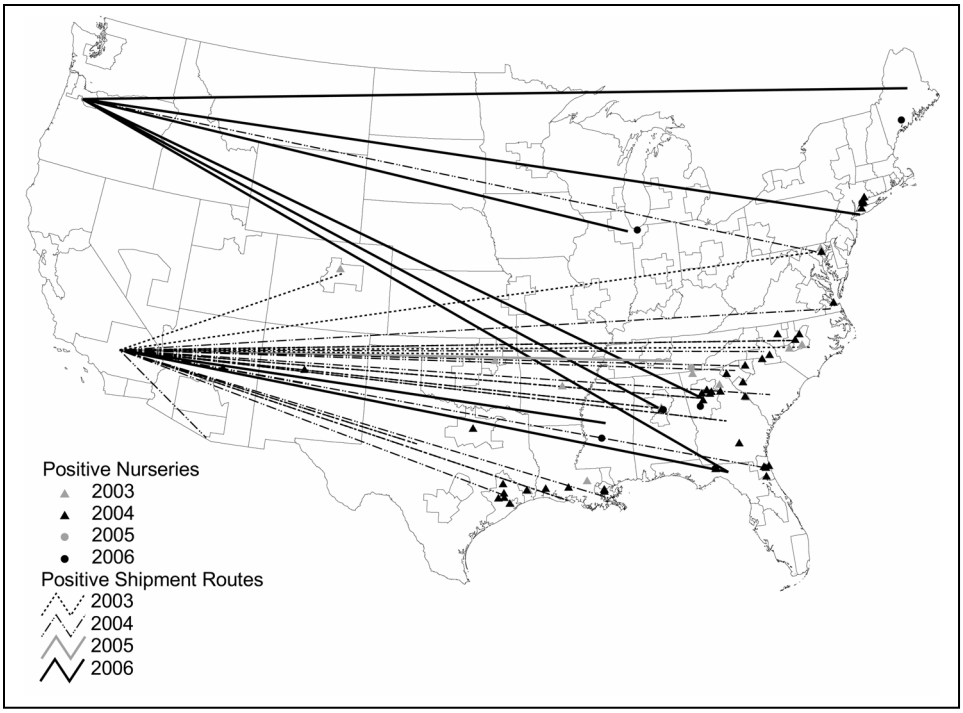

Figure 2—U.S. confirmed *P. ramorum* infected host plant shipments.

Model Outline

The scenario studied here involves a short time period in which new infections by *P. ramorum* are detected in previously pristine sites. We assume that all the newly detected infections result from nursery stock shipped from a single previously infected source. The goal of the model is to assign a probability to each source indicating the likelihood that a source is the sole origin of the infection. Once these conditional probabilities are determined, probabilities of undetected infection can be assigned to all other susceptible sites, allowing managers to focus subsequent inspection efforts on sites most at risk of *P. ramorum* infection.

To this end, a directed bipartite network is defined with source nodes corresponding to known domestic sources of *P. ramorum* infected nursery stock and destination nodes corresponding to sites that receive nursery stock from infected sources. Nursery stock flows along the links of this network, each of which leads from a source node to a destination (or site) node. The flow rate along each link equals the amount of nursery stock flow, by weight, carried from the source to the destination.

A standard Bayesian probabilistic model is imposed on this network to update probabilities of each source node as being the origin of recently found new infections. A priori probabilities of being the solitary origin of *P. ramorum* infection can be assigned to each source based on many factors, including historical observations, rate of importation from possible foreign sources of *P. ramorum* and other relevant factors. Which factors are most relevant will become clearer as the biology and ecology of *P. ramorum* becomes better understood.

Probabilities of transmission are also assigned to each link based on nursery stock flow rates along the link. With these probabilities determined, a simple application of Bayes' Rule can serve to update the probabilities that a given source site is the unique origin of the newly uncovered infection pattern.

Model Notation

Here we begin the formal description of our model. Let

$$S = \{s_1, s_2, \ldots, s_i, \ldots, s_m\}$$

be the set of nodes corresponding to potential sources of *P. ramorum* infection. These sites will be areas of known *P. ramorum* infection that export significant amounts of relevant nursery stock. Similarly, let

$$D = \{d_1, d_2, \ldots, d_j, \ldots, d_n\}$$

be the set of nodes corresponding to destinations of nursery stock shipments. At the beginning of the model's time period these destinations are assumed to be free from *P. ramorum* infection.

To reflect the results of the destination inspections carried out during the time frame of the model, define D_I to be the set of destination nodes found to be infected, define D_C to be the set of destination nodes found to be "clean," in other words, uninfected, and let D_U be the set of destination nodes for which infection status is unknown. The union of these three sets is D, the set of all destination nodes.

Let E be the event that the infection pattern determined by the sets D_I, D_C and D_U has developed during the model time period.

Furthermore, let F_i be the event that source s_i is the sole origin of the observed infection pattern. The assumption that exactly one source caused the observed infection pattern implies that the events F_i are disjoint and together cover the universe of outcomes.

The flow rates of nursery stock play an important role in our model. To this end, let f_{ij} be the flow of stock from source s_i to destination d_j.

Lastly, we will need to compute probabilities that a flow rate from a specific source to a specific destination results in the initial infection of that destination during the model time period. This computation will be based upon a parameter p defined to be the probability that a single unit of flow will result in the initial infection of the flow's destination.

Ranking Sources as Origins of Infection

The first goal of our analysis is to determine, for each source s_i, the probability that s_i is the origin of the observed infection pattern. In terms of our notation what we seek

is the conditional probability $P(F_i|E)$. By a direct application of Bayes' Rule we see this probability can be computed as

$$P(F_i \mid E) = \frac{P(E \mid F_i)P(F_i)}{\sum_{k:s_k \in S} P(E \mid F_k)P(F_k)} \quad (1)$$

for every i such that $s_i \in S$.

To use (1) we need values for $P(E|F_i)$ and $P(F_i)$ for every source s_i. The probabilities $P(F_i)$ are the a priori probabilities of each source being the origin of infection. These probabilities are assigned by users of the model based on historical observations, rates of importation from possible foreign sources of *P. ramorum* and other relevant factors. It should also be noted that the $P(F_i)$ terms in (1) can be replaced by nonnegative relative likelihoods, quantities that need not sum to precisely one, without affecting the validity of the expression.

The probabilities $P(E|F_i)$ of achieving the observed infection pattern given s_i is the origin of the infection are calculated from nursery stock flow rates and an assumption of independent destination infections. Recall that p denotes the probability of a single unit of flow infecting a previously uninfected destination. If f denotes the flow from some infected source to some destination, the probability of the infection of the destination is given by

$$P(\text{new infection given flow } f) = 1 - (1 - p)^f. \quad (2)$$

Assuming that infection of different destinations are independent events, we can now derive the conditional probability $P(E|F_i)$ for each source s_i. Recalling that f_{ij} denotes the flow of nursery stock from source s_i to destination d_j during the model time period, we see

$$P(E \mid F_i) = \left[\prod_{j:d_j \in D_I} (1 - (1 - p)^{f_{ij}}) \right] \left[\prod_{j:d_j \in D_C} (1 - p)^{f_{ij}} \right]. \quad (3)$$

The first term on the right hand side of (3) represents the probability of all the infections that were realized. The second term gives the probability of all the non-infection events. The destinations for which no information is available, the elements of D_U, play no role in this computation.

At this point we are able to compute all the quantities required by (1) to compute the conditional probabilities for each source. Doing so allows us to rank the sources according to the probability that they were the origin of the new infection pattern. The updated probabilities also allow us to identify destinations from the D_U set that are at high risk of having also been infected during the model time period.

Identifying High Risk Destinations

Assigning probabilities of infection to each destination in D_U can be achieved by the common technique of partitioning the universe of possibilities into a small number of disjoint events. In our context the relevant partition comprises the events $F_i|E$ for each source s_i. This gives us

$$P(\text{site } d_j \text{ is newly infected}|E) = \sum_{i:s_i \in S} P(\text{site } d_j \text{ is infected by source } s_i|E \cap F_i)P(F_i|E)$$
$$= \sum_{i:s_i \in S} (1-(1-p)^{f_{ij}})P(F_i \mid E) \quad (4)$$

where the probabilities $P(F_i|E)$ can be computed according to (1).

Application to Large Networks

To be of practical use this model must be applicable to moderately large networks. Using custom software, written by McKelvey in the Java programming language, the model above has been applied to the nationwide network of nursery stock flows described in the Introduction. This network consists of nine regional sources in California, Oregon and Washington and 101 destination regions throughout the country (see figure 3). On this network the software performed all the calculations seemingly instantaneously.

The number of operations required to perform the computations in this model grows as the number of nodes and links in the network. If m is the number of sources in the scenario and n is the number of destinations, the time and memory necessary to perform all the calculations is bounded from above by a number proportional to $m+n+mn$. Thus, for large networks, a doubling in the size of the network increases the upper bounds on the time and memory needed for the computation by roughly a factor of four. This quadratic growth indicates that scaling issues are unlikely to pose serious impediments to applying the model to large scale problems.

Figure 3—U.S. national stock flows.

Future Research

Future research will be directed toward loosening the independence assumptions and the more problematic assumption that the entire newly observed infection pattern has precisely one source as its origin. While these assumptions lead to a model that is useful in relating nursery stock flow patterns and recent infection activity to the risk of future infection events, the current methodology fails to exploit patterns in the commodity flow or the possibility of several concurrent infection origins. In addition, the possibility of infection introduced at transhipment points is not addressed currently. All of these issues will be addressed in future work.

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Epidemiological Modeling of *Phytophthora ramorum*: Network Properties of Susceptible Plant Genera Movements in the Nursery Sector of England and Wales[1]

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Abstract

Since the first finding of *Phytophthora ramorum* in the U.K. (on *Viburnum tinus*, 2002), the pathogen has been reported throughout the country on a variety of susceptible species both in the horticultural sector and in woodlands and historic gardens. The nursery network may have properties which affect the epidemic threshold for spread of the pathogen. Here, as part of ongoing modeling studies, we provide an analysis of the database of positive *P. ramorum* records in England and Wales (2003 to 2005), both in terms of affected plant genus and country of origin. The majority of positive findings are reported on *Rhododendron* (68 percent), with a substantial minority on *Viburnum*, *Camellia*, and *Pieris*. Another 15 genera make up the remaining 3 percent of positive records. These findings highlight the wide range of plant species at risk from the pathogen, and suggest that *Rhododendron* could act as a super-connected susceptible genus. This property would be characteristic of a scale-free network. As for the country of origin, the majority of findings originate from the U.K., but a non-negligible number (~ 30 percent) of positive records originate from several other countries. Models of network movements of plants susceptible to *P. ramorum* therefore need to take into account that this is a largely open system, with both links in and out of a single country. The number of *P. ramorum* susceptible plant species and the number of countries involved in their trade have certainly facilitated spread and pose a formidable challenge to the current effort to eradicate or contain the pathogen.

Key words: Horticultural trade, landscape pathology, networks epidemiology.

'*Phytophthora*' is Greek for plant destroyer, whilst '*ramorum*' is Latin for branched, referring to the pathogenicity of *P. ramorum* to twigs and branches of *Rhododendron* and other shrub species (Werres and others 2001). As such, *P. ramorum* would be relevant to studies in network epidemiology if only because of its name. However, this pathogen has been reported from plant nurseries in several countries (for example, Werres and others 2001; Appiah and others 2004; Parke and others 2004;

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Stokstad 2004; Herrero and others 2006; Tubajika and others 2006) and these nurseries form a complex directed trade network by exchanging plant material.

Networks are becoming increasingly relevant to epidemiology (for example May 2006). This is because networks such as that in global aviation can efficiently spread human diseases across the world (for example Hufnagel and others 2004). This happens for a number of reasons (for example Guimerá and others 2005): (i) the global aviation network is a giant component, which means that it is possible to fly from any airport in the world, even if not directly, to any other airport; (ii) in many cases, this is possible with a relatively low number of flights, due to the presence of international hubs, or super-connected airports; (iii) moreover, there is a high heterogeneity in the areas covered by the network, and in the strength of the links.

Networks are relevant not only to epidemiology (for example, Newman 2003). In fact, there has been an upsurge of studies applying the tools of network theory to a number of disciplines, from food web ecology to sociology and computer science (for example, Amaral and others 2000). If we attempt to bring some order to this burgeoning material according to the degree to which the analyzed networks are natural, social or technological, it is clear that epidemics have each of these characteristics: disease spread is a natural phenomenon, it is facilitated by technology (although technology also helps in preventing disease spread), and, since people are involved, epidemics are also a social issue.

Networks are sets of elements connected by links. In the context of the networks underlying the spread of *P. ramorum*, nodes can be the nurseries trading susceptible species, and links can be shipments of these plants between nurseries. Nodes can be connected in various ways, and this leads to a typology of networks (for example, Dybiec and others 2005, Keeling and Eames 2005). Local networks are modeled as more or less regular lattices. Their feature is neighboring connectivity. If we retain this neighboring connectivity but add the rewiring of some local links into shortcuts, or long-distance connections, we obtain small-world networks. If nodes are connected to other nodes randomly, in other words with a certain probability p, then the network is random. Scale-free networks are characterized by the presence of hubs, or super-connected individuals.

It turns out that, other things being equal, the structure of networks can have a profound effect on epidemic development. Simple models of disease spread in networks with constant number of nodes and links and with epidemic starting from a single infection show that the disease steady-state is first reached in scale-free networks, then in random ones, and only later in small-world and local networks (Shirley and Rushton 2005). For nurseries trading plants susceptible to *P. ramorum* or other pathogens, a SIS (Susceptible-Infected-Susceptible) model may be more realistic than the more conventional SIR (Susceptible-Infected-Removed) model (for example, Gilligan and others 1997, Jeger and others 2007). Even if lessons are learnt from past errors and best management practices are adopted, nurseries where the pathogen has been detected are still at risk of becoming infected after the pathogen has been eradicated if they continue to handle plant species susceptible to *P. ramorum*.

Measures aiming at containing the spread of *P. ramorum* in nurseries are being taken in the U.K., and there is evidence that this containment policy is effective. Figure 1

shows the temporal development of the number of positive records to *P. ramorum* in nurseries and garden centers of England and Wales (2003 to 2005; data kindly provided by the Department for Environment, Food, and Rural Affairs, U.K.; the database has on the whole 2,788 positive records to *P. ramorum* out of 81,036 tested records). As a site can have more than one positive record (because *P. ramorum* may have been found on different species and on different dates), this graph is not the same as that for positive nursery and retail centre sites shown by David Slawson (this Proceedings), although in both cases there is a downward trend with time.

Relevant to a network perspective is information on whether or not these positive records are thought to have originated in the U.K. This information is needed to fill the knowledge gap about the long-distance and inter-country spread of *P. ramorum* infection (Appiah and others 2004). The origin of the pathogen on the plants can never be stated with certainty - however, where it is suspected that the country that dispatched the plants also dispatched them with an infection of *P. ramorum*, this is notified by the Department for Environment, Food and Rural Affairs (U.K.) to the European Commission, the sending country, the EU member states and EPPO. Although there is variation from month to month, *Figure 2* shows that a substantial proportion of positive findings are thought to originate from abroad. For example, 106 out of 244 positive findings in 2003 (43 percent) in nurseries were on plants of non-U.K. origin. The proportion is similar in 2005 (38 out of 100 findings), in spite of a reduction in the overall number of findings, which suggests that the policy for

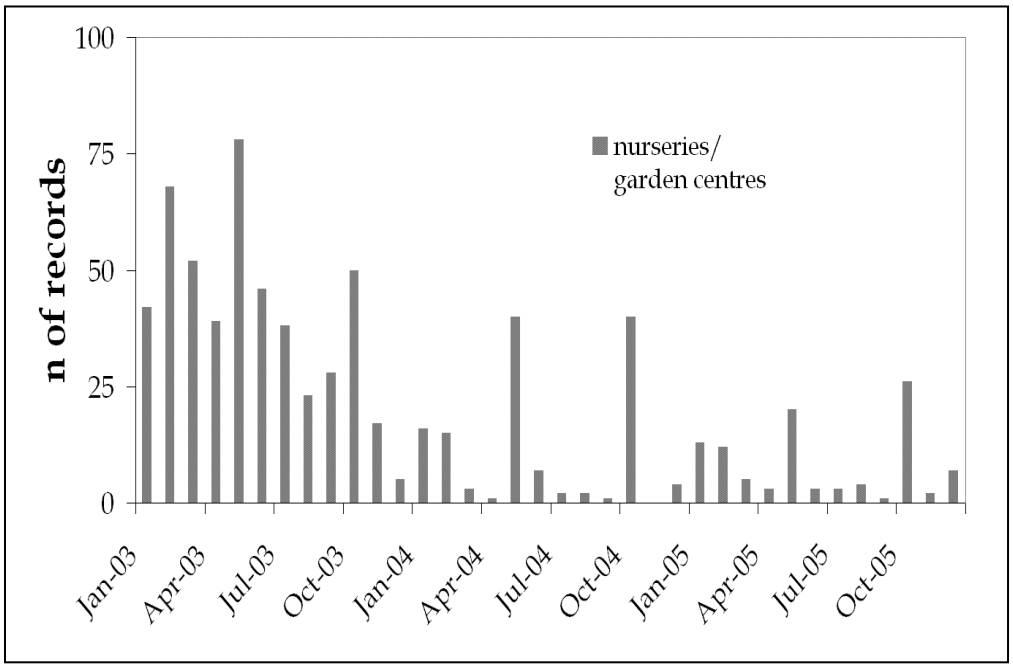

Figure 1—Downward trend of the records positive to *P. ramorum* in nurseries and garden centres (2003-2005) of England and Wales (data source: DEFRA, U.K.).

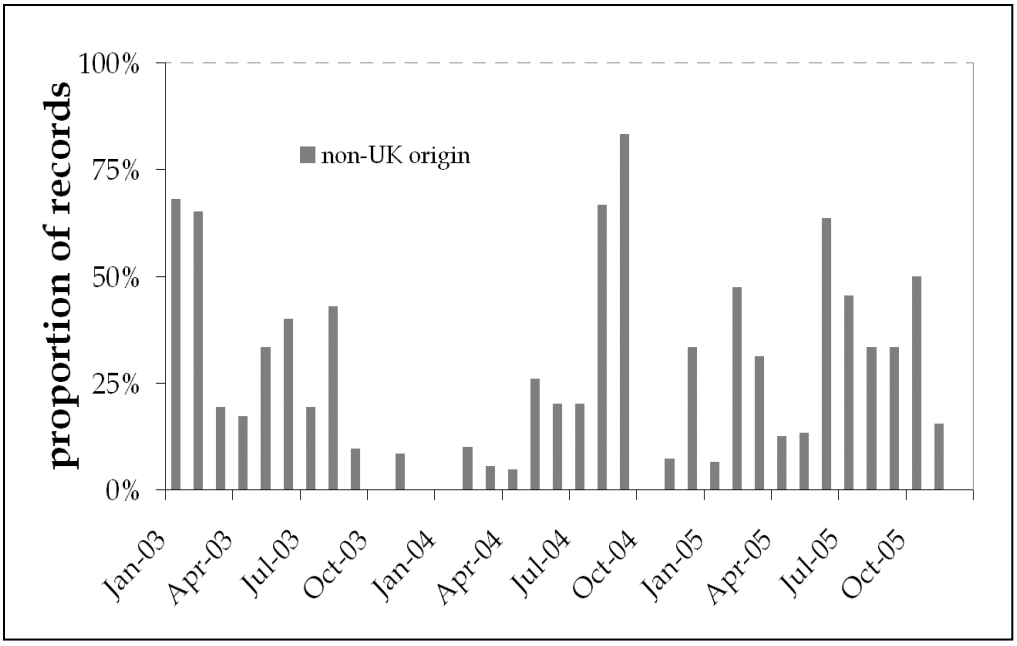

Figure 2—The proportion of *P. ramorum* positive records originating from the U.K. or from other countries (same period and source as fig. 1, but for nurseries only).

dealing with internal disease transmission is working more effectively than the policy for dealing with imports from outside of the U.K. All in all, this is evidence for a small-world scenario in the spread of *P. ramorum*, with local connectivity but also long-distance connections.

Moving from a temporal to a spatial perspective, the distribution of positive findings in woodlands and historic gardens (Fig. 2 in Jeger and others 2007) appears to match the climate suitability of Britain to *P. ramorum* (Appendix 3 in Forestry Commission 2004). Also at a large-scale, disease expression is the result of the interactions amongst host(s), pathogen and environment (Holdenrieder and others 2004, Rizzo and others 2005, Scholthof and others 2007). Since *P. ramorum* hosts (as well as their associations) are fairly widespread, and since the pathogen has been detected in nurseries and garden centres throughout Britain, climate may be the limiting factor restricting disease expression in woodlands and gardens mainly in the West. However, this assumes that the pathogen was first spread by the nursery trade and then moved into woodlands and historic gardens. If instead the pathogen first became established in historic gardens and/or woodlands and then jumped into the nursery trade, the network of plant nurseries has the potential to spread the pathogen also to regions where there are currently no or few outbreaks in woodlands and/or historic gardens. In this perspective the downward trend of *P. ramorum* positive findings in nurseries is to be welcomed. Less encouraging is the increase in the number of positive records in woodlands and historic gardens (Judith Turner, this proceedings).

A network perspective can be applied to the database of *P. ramorum* positive findings also at the level of the different susceptible genera (genera and not species, because for many positive findings only the generic information is available), similar to analyses of mycorrhiza (Southworth and others 2005) and plant-frugivore

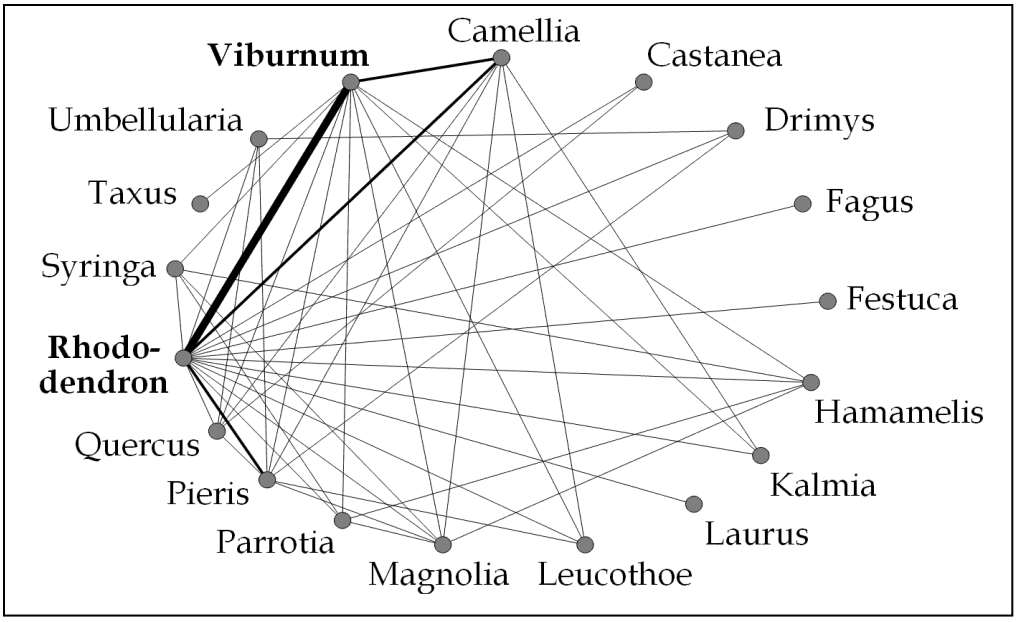

Figure 3—Web of genera susceptible to *P. ramorum*. Two genera are connected if *P. ramorum* was detected on individual plants of these genera at the same location (2003-2005, England and Wales, nursery, garden centers and natural environment, data provided by DEFRA, U.K.). The connection strengths reflect the number of locations connecting the two genera (*Rhododendron-Viburnum*: 55 common locations, *Rhododendron-Camellia*: 17, *Rhododendron-Pieris*: 16, *Viburnum-Camellia*: 10, all other combinations: less than 10 common locations).

networks (Lázaro and others 2005). In this case, two genera are connected if individuals of the genera are found to be infected by the pathogen at the same location (Fig. 3). The resulting network is not homogeneous: some genera are more connected than others. This could be a consequence of *Rhododendron ponticum* being the main host in Britain (68 percent of the positive findings of *P. ramorum* are on *Rhododendron*). This finding supports the current policy focus on high risk species. By targeting locations where super-connected genera occur (in other words *Rhododendron* and *Viburnum*), it is possible to cover most if not the whole of the *P. ramorum* host range.

Also in terms of the number of *P. ramorum* positive records for the different genera, there is evidence for a high heterogeneity of the pathosystem. The frequency distribution of the number of affected genera in terms of the number of positive *P. ramorum* records per affected genus is not log-normal (fig. 4). What one would expect would be a normal distribution, in other words a few genera with a small amount of positive records, a few genera with a large amount of positive records, and most genera with an intermediate number of records. But what we observe is not a normal distribution, as most genera have a few positive records, and only a few genera have many positive records. In fact, this distribution is relatively well fitted by a linear regression in log-log space. This is evidence for the presence of a scale-free property in the database of *P. ramorum* positive findings in England and Wales. Scale-free means that the decrease in the order of magnitude of affected genera with increase in order of magnitude of number of positive records per genus is roughly

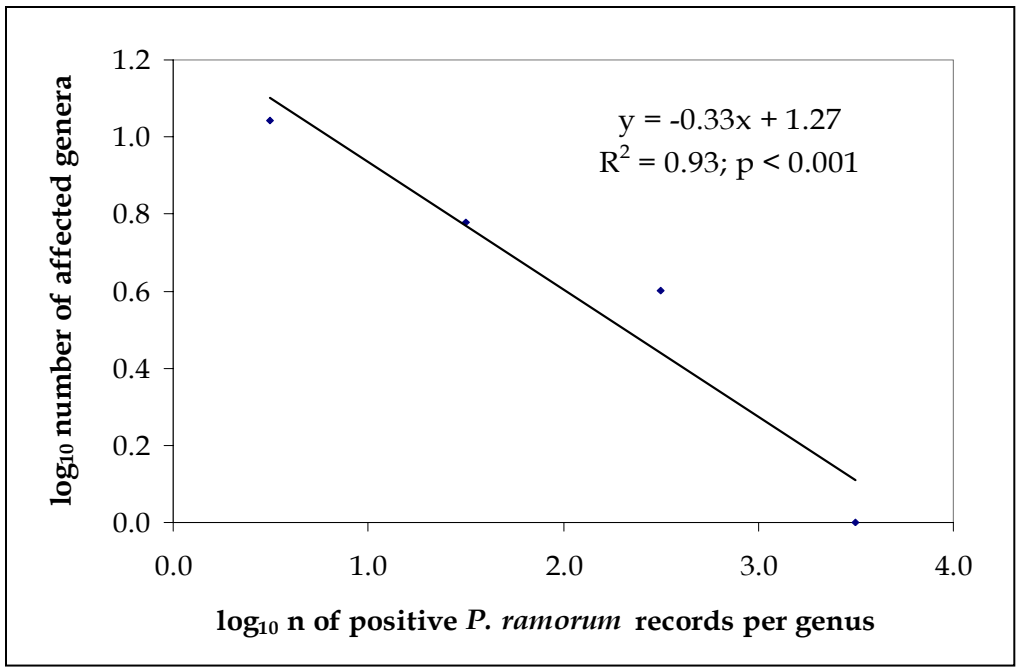

Figure 4—Linear regression of the distribution of the number of affected genera in logarithmic classes of the number of positive *P. ramorum* records (2003–2005, England and Wales, nurseries, garden centres and natural environment, data provided by DEFRA, UK).

similar over the whole range of variation in affected genera and positive records. For a friendly introduction to scale-free networks see for example, Southworth and others (2005). Further work should aim to establish whether the nursery network underlying the spread of *P. ramorum* indeed has a scale-free degree distribution. The degree distribution is the frequency distribution of the number of links of the nurseries involved in the trade of plants susceptible to the pathogen. Unfortunately, this level of information about the structure of the U.K. horticultural trade is not currently available.

The findings that the network involved in the spread of *P. ramorum* in England and Wales is likely to have small-world and scale-free properties is worrying, because such networks have lower epidemic thresholds than local and random networks. However, scale-free networks may improve the chances of preventing further increase in the extent of the regions affected. Provided that long-distance connections and key players in the nursery trade can be identified, by targeting them instead of pursuing complete or random control of nurseries and shipments, it is possible to achieve a more efficient and less costly reduction of the connectivity of the system, as shown by studies of the vulnerability of the North American power grid to random vs. targeted removal of power stations (Albert and others 2004).

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Assessment of Potential Economic and Environmental Impacts Caused by *Phytophthora ramorum* in Europe[1]

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Abstract

Economic and environmental impacts of *Phytophthora ramorum* in Europe were evaluated within the European Union framework 6 project on "Risk Analysis for *P. ramorum* a pathogen threat to Europe" (RAPRA). Impact assessment was conducted according to three different scenarios: 1. "Nursery System" - describes losses occurring in nurseries where special growing conditions and trade with plant material influence spread and dimension of the disease, 2. "Northern European Tree Host System" - based on observations in the United Kingdom, the Netherlands and Germany where infections of trees (beech, red oak) at forest or park sites only occur, when *Rhododendron* as a foliage host is present to a certain extent and 3. "Southern European Tree Host System" where a potential foliage host is *Quercus ilex* growing as an understory plant in combination with susceptible tree hosts. Taking climatic conditions favourable for the pathogen, distribution of host plants and pathogen distribution into account, the present economic and environmental impact in Europe is minimal to moderate. Potential impacts depend on the host system: for nurseries, facing higher costs for hygiene measures, treatments of plant material and trade impacts, no changes are expected in the future as long as basic conditions like plant health regulations are not altered noticeable. In the "Northern European Tree Host System" present impacts are moderate and restricted to few areas where *Rhododendron* are associated with tree hosts and environmental impact is caused. The potential impact is expected to be not more than moderate as long as no widely distributed foliar hosts of Northern European forests occur. In the "Southern European Tree Host System" present impacts are minimal since the pathogen does not occur in the environment. But here potential impact is assumed major if *P. ramorum* would be introduced and spread in the unique Mediterranean laurel and *Q. ilex* forests.

Key words: *Phytophthora ramorum*, sudden oak death, economic impact, environmental impact, plant health.

Introduction

Potential impacts caused by *Phytophthora ramorum* in Europe were assessed within the European Union (EU) framework 6 project on Risk Analysis for *P. ramorum*, a recently recognized pathogen threat to Europe and the cause of Sudden Oak Death in the United States (U.S.) (RAPRA, www.rapra.csl.gov.uk). Impacts are to be expected where climatic conditions are favorable for the development of the pathogen and susceptible host plants are present. This comes on the one hand true for nurseries

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growing host plants with optimized growing conditions for the pathogen. For the natural environment on the other hand there are different areas in Europe more or less suitable for the pathogen, depending on the distribution of host plants and climate data. In order to cover the most important scenarios, three different impact scenarios were assumed (see also Steeghs 2007):

1. The **Nursery System** describes losses occurring in nurseries where special growing conditions and trade with plant material favour spread and dimension of the disease.

2. The **Northern European Tree Host System** with *Rhododendron* as a foliar host next to tree hosts (for example beeches) which is based on observations in the United Kingdom (U.K.), the Netherlands and Germany where infections of trees (beech, red oak) at forest or park sites only occur, when *Rhododendron* as a foliage host is present to a certain extent where the pathogen produces enough inoculum.

3. The **Southern European Tree Host System** with maquis and heathland plants growing as understory plants in combination with susceptible tree hosts with *Quercus ilex* as a potential foliage host where the pathogen produces enough inoculum.

Results and Conclusions

Based on the three scenarios the present economic and environmental impacts caused by *P. ramorum* in Europe are minimal to moderate (Table 1). Nurseries are facing higher costs especially for additional hygiene measures, treatments of plant material and impacts on trade with host plants of *P. ramorum*. These impacts are not expected to change in the future as long as basic conditions (like plant health regulations) are not altered noticeably. In the Northern European Tree Host System present impacts are moderate. They are restricted to few areas where rhododendrons are associated with tree hosts (special areas in the Netherlands and Great Britain). In these areas environmental impacts were caused to a certain extent. The potential impact for Northern European areas at risk is expected to be not more than moderate as long as no other foliar hosts with potential for high inoculum production in the forests and parks occur. In the Southern European Tree Host System present impacts are minimal since the pathogen does not occur in the environment. But potential impact is assumed major if *P. ramorum* would be introduced into the Mediterranean laurel and *Quercus ilex* forests. Climatic conditions for establishment and spread of the pathogen in these regions are very favourable according to R. Baker, 2006, Central Science Laboratory, U.K., member of RAPRA consortium, unpublished climate matching results for *P. ramorum*).

Table 1—Present and potential impacts of *Phytophthora ramorum* in Europe for three different host plant systems (scenarios)

Scenarios	Present Impact[1]	Potential Impact[1]	Likelihood[2]
Nurseries	moderate	moderate	very likely
Northern Europe	moderate	moderate	likely
Southern Europe	minimal	major	possible

[1] rating: minimal, minor, major, moderate, massive (according to DEFRA 2005)

[2] rating: very unlikely, unlikely, possible, likely, very likely (according to DEFRA, 2005)

Compared to the Sudden Oak Death situation in the U.S., Europe is facing - up to now - a very different impact situation: impacts due to *P. ramorum* in Europe are less severe and very restricted so that containment measures can help to prevent spread to other European regions at risk (like Mediterranean areas with susceptible tree hosts and favourable climate). The benefit of the phytosanitary measures has to be evaluated within a cost-benefit analysis.

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Climate-Host Mapping of *Phytophthora ramorum*, Causal Agent of Sudden Oak Death[1]

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Abstract

We modeled *Phytophthora ramorum* infection using the North Carolina State University-Animal and Plant Health Inspection Service Plant Pest Forecasting System (NAPPFAST) for the conterminous United States. Our infection model is based on a temperature-moisture response function. The model parameters were: leaf wetness, minimum temperature, optimum temperature and maximum temperature over a specified number of accumulated days. The NAPPFAST weather database, which involves approximately 2,000 weather stations across North America, was used to generate a spatially interpolated climate match for *P. ramorum* infection at a 10 km^2 resolution. The model was used to create maps showing the frequency of favorable years for infection, which were validated by comparison with historical *P. ramorum* observations from California in urban and natural settings. We then overlaid climate match areas for *P. ramorum* infection with hardwood forest density and understory host density to generate a composite risk map. We further increased the precision of the maps by applying masks that removed areas where: 1) no climate match occurred, 2) lethal cold soil temperatures for *P. ramorum* occurred, 3) no hardwood hosts occurred and 4) no understory hosts occurred. These maps are designed to improve the efficacy and economy of survey and detection activities for *P. ramorum* by federal, state and local regulatory agencies. We also generated global maps using the NAPPFAST system at a 55 km^2 monthly resolution. Parameters used in the global model were: minimum average monthly temperature, maximum average monthly temperature and monthly precipitation. We used an air temperature lethal cold mask as a surrogate for soil temperature in the global model. These maps may assist in the search for the origin of *P. ramorum*.

Key words: Infection, validation, climate, hosts, risk map.

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Introduction

Phytophthora ramorum, the causal agent of sudden oak death, is currently found in localized areas of California and Oregon (USDA-APHIS 2007). *Phytophthora ramorum* has caused considerable oak mortality in California and is undergoing eradication in Oregon. There are concerns that *P. ramorum* could be introduced into the eastern United States where it could threaten indigenous hardwoods.

To assist in activities like early detection, survey, mitigation and formation of regulatory policy, we generated risk maps that visualize areas at risk for *P. ramorum* infection for the conterminous United States. Our maps were generated from models incorporating climatology and host presence. We also generated global maps for frequency of infection occurrence to assist in the search for *P. ramorum's* origin.

Materials and Methods

Conterminous United States Model

Climatology—

We used the North Carolina State University-Animal and Plant Health Inspection Service Plant Pest Forecasting System (NAPPFAST) to simulate the climatology models (Borchert and Magarey 2004, Magarey and others 2007). NAPPFAST is an internet-based modeling tool with flexible templates for model creation. Our models are based on historical climate data for the past 10 years. We generated both monthly and annual maps for frequency of infection occurrence. In constructing our models, we assumed that *P. ramorum* would be climatically limited by moisture and temperature requirements for zoosporic infection.

We generated a North American *P. ramorum* infection model at a 10 km^2 spatial and daily temporal resolution. The spatial interpolation is based upon a weighted average (Barnes, 1964) and generated from commercial and government weather station data utilizing approximately 2,000 weather stations in North America (Russo, 1999). Daily leaf wetness hours and soil temperature are derived using proprietary algorithms. Validation of the leaf wetness algorithm has been published (Magarey *et al*., 2005). The weather data undergoes quality control prior to archiving.

The infection model is based on a temperature response function that is scaled to a daily leaf wetness requirement (Magarey *et al*., 2005). The model inputs were daily average temperature and daily total hours of leaf wetness for each of the past 10 years. The model parameters were: 1) minimum daily temperature ≥ 3 °C, 2) optimum daily temperature of 20 °C, 3) maximum daily temperature ≤ 28 °C and 4) ≥ 12 hours of accumulated leaf wetness per day (Hüberli *et al*., 2003, Orlikowski and Szkuta 2002, Tooley and Kyde 2005, Werres *et al*., 2001). For each day the model returns a value between zero and one. These values are then accumulated over time. We used a threshold of ≥ 10 accumulated days for the monthly maps and ≥ 60 accumulated days for the annual map (Jones personal communication, 2004). The climate risk map represents the frequency of years in 10 meeting these conditions.

Climate Model Validation

We used two confirmation data sets to validate the climate model. The first was California OakMapper, which reported detections in California as of December 20, 2006 (UC Berkeley, 2006). The second consisted of field confirmations in Sonoma County, California (Meentemeyer personal communication, 2006).

Hosts

We obtained the hardwood host layer from the National Land Cover Dataset (NLCD) for the conterminous United States (Vogelmann *et al*., 2001). This data layer expresses forest composition in percentages. We assumed that mixed forests would have an equal distribution of hardwoods and softwoods. We queried the NLCD data set for all hardwoods plus 50 percent of mixed forests to generate the hardwood host layer. This raster was visualized at a one km^2 resolution.

We obtained the understory host layer from NatureServe (2002). We estimated the distribution of seven Ericaceae hosts of *P. ramorum* and combined them as a shapefile that was demarcated based on frequency of occurrence. This was then converted to a raster at a one km^2 resolution. We converted this raster to percentages by dividing it by seven and multiplying by 100 using the raster calculator in ArcGIS.

Masks

We used four masks in our climate-host risk map for the conterminous United States at a one km^2 resolution. We masked out areas where: 1) no climate match occurred, 2) no hardwood hosts occurred, 3) no understory hosts occurred and 4) a lethal cold soil temperature of –25 °C occurred for at least one day during January (DEFRA, 2004).

For the global infection model we cannot currently map soil temperature. Consequently, we used an average minimum monthly air temperature of –10 °C as a surrogate for the –25 °C lethal cold soil temperature mask that was used in the map for the conterminous United States. We masked areas where the average minimum monthly temperature was less than –10 °C for at least one month during the year.

The NAPPFAST one-year climate-match frequency band identifies areas that have a match of one and below. Consequently, this band is visualizing areas that are not at risk for *P. ramorum* infection. To account for this, we used the zero and one-year bands to mask areas where no climate-match occurred.

Risk Model

We generated the risk map by summing the percentage values for climate frequency, hardwood hosts and understory hosts and dividing the total by three. We then applied the four masks to remove areas where infection could not occur.

Global Climate Model

The global infection model was generated at a 55 km^2 spatial and monthly temporal resolution (Climate Research Unit, Norwich, U.K.) (New *et al*., 1999). The model parameters were: 1) average minimum temperature ≥ 3°C, 2) average maximum temperature ≤ 28 °C, 3) ≥ 10 days of precipitation during the month and 4) ≥ 2

months meeting these conditions. The risk map represents the frequency of years in ten meeting these conditions.

Results and Discussion

All of the *P. ramorum* confirmations for both validation data sets occurred in the highest climate frequency of occurrence band (figs. 1 and 2). Although the validation is based on confirmation data from one state, it suggests that our model is accurately predicting where infection will occur.

The risk maps indicate that both the west coast and the eastern third of the conterminous United States, especially the Appalachian Mountains, are at similar risk for infection (fig. 3). The relative risk maps also allowed for precise visualization of at-risk areas for infection and establishment (fig. 4). This information can aid in surveys, early detection and eradication of the disease should it move east.

Our global prediction model identified areas at greatest risk for infection on each continent, though at a coarse resolution (fig. 5). Future iterations of the NAPPFAST system will generate global predictive maps at a 10 km^2 daily resolution. These higher resolution maps will be of greater use in the search for *P. ramorum's* origin and also provide a means for additional validation by comparing our model's predictions with known disease occurrence in other countries.

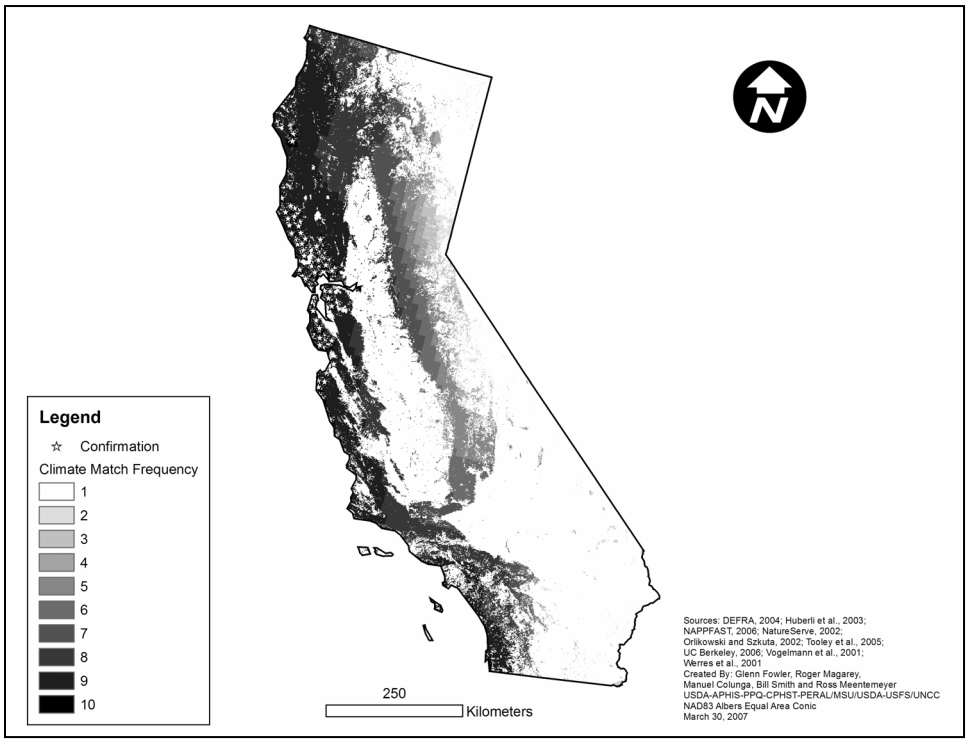

Figure 1—Infection model validation using the OakMapper data set.

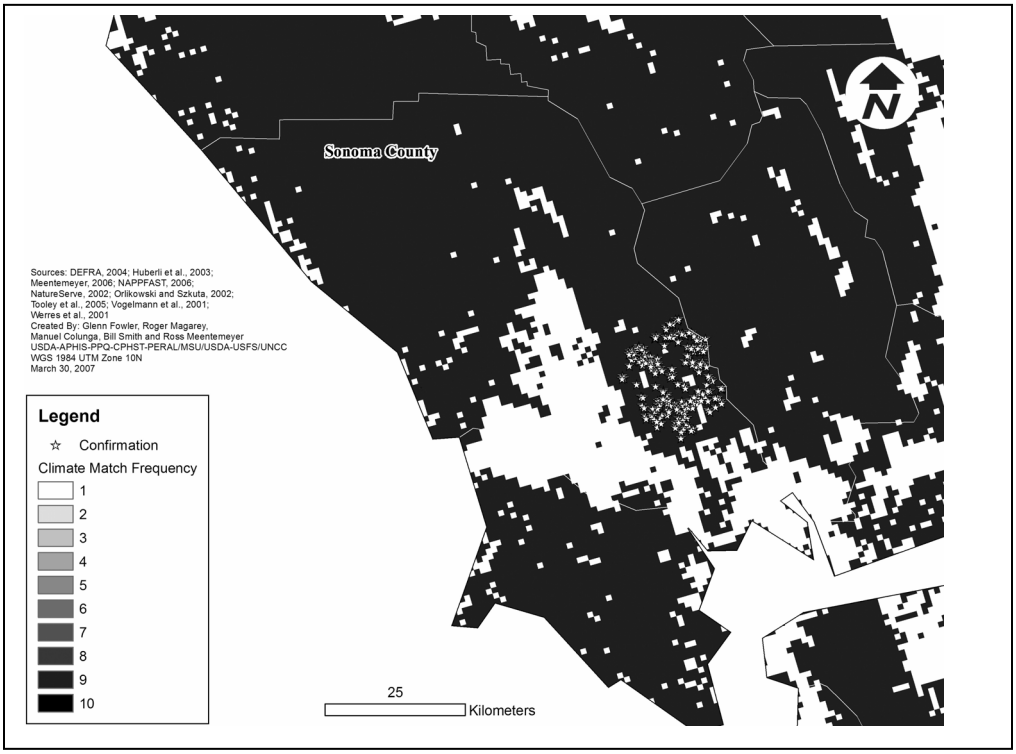

Figure 2—Infection model validation using the Meentemeyer (2006) data set in Sonoma County, California.

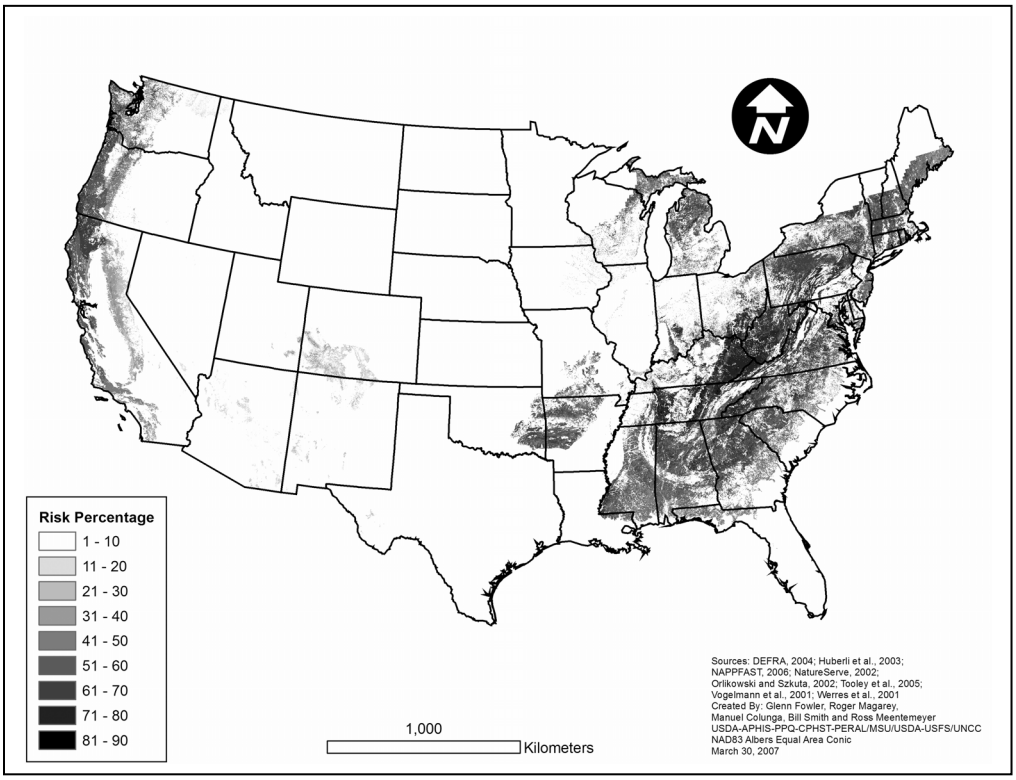

Figure 3—*Phytophthora ramorum* infection risk map for the conterminous United States based on climate and host data.

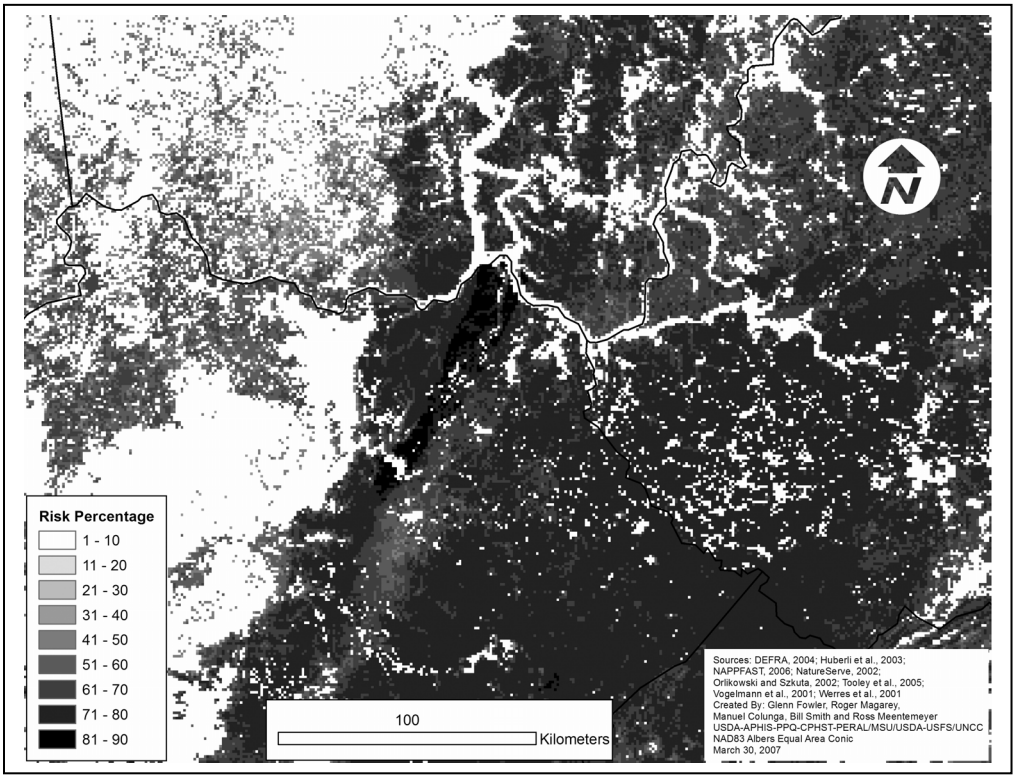

Figure 4—Precise visualization of at-risk areas for *P. ramorum* infection.

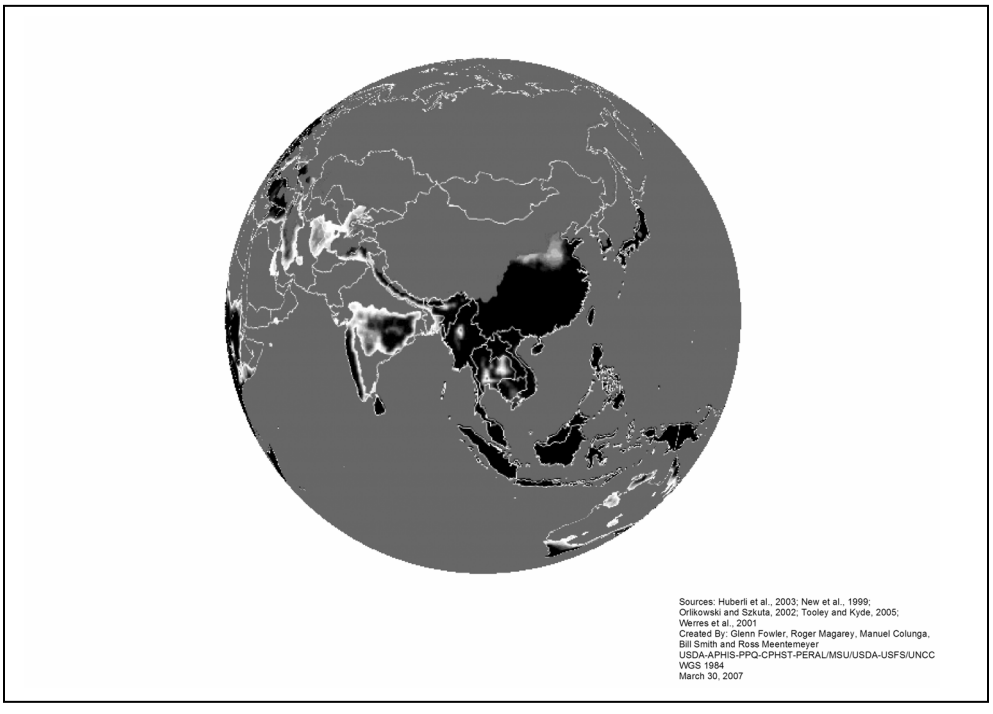

Figure 5—Sample global risk map visualizing areas with suitable climates for *P. ramorum* infection.

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Predicting the Spread of Sudden Oak Death in California: Spatial-Temporal Modeling of Susceptible-Infectious Transitions[1]

Richard D. Hunter,[2] Ross K. Meentemeyer,[2] David M. Rizzo,[3] and Christopher A. Gilligan[4]

Abstract

The number of emerging infectious diseases is thought to be increasing worldwide - many of which are caused by non-native, invasive plant pathogens I n forest ecosystems. As new diseases continue to emerge, the ability to predict disease outbreaks is critical for effective management and prevention of epidemics, especially in complex spatially heterogeneous landscapes. Mathematical modeling of susceptible-infectious transitions in plant epidemics often incorporate spatial dynamics, but are rarely applied to real-world landscape data using geographic information systems (GIS). In spite of the challenges, application of epidemic models to GIS data provides several opportunities, including the ability to map high risk locations for management and early detection of disease outbreaks, assess ecological and economic impacts of disease in specific geographic regions, and visualize potential outcomes of management actions. Here, we present a spatial-temporal model of the spreading forest pathogen *Phytophthora ramorum*, causal agent of sudden oak death. Our goal was to develop a spatially-explicit epidemic model that predicts susceptible-infectious (SI) transitions at discrete time steps using a geographic cellular automata approach. To increase understanding and guide management, we examined 1) the ability of the modeling approach to predict the geographic distribution of disease spread, 2) the degree to which rates of disease spread vary through time and space, and 3) the influence of stochastic variability on epidemic outcomes. We first describe a generic mathematical model for a susceptible-infectious epidemic that simulates spatial and temporal patterns of disease spread on a weekly time step for application at large spatial scales. Next, we describe how data from field and lab studies were used to parameterize the driving system variables, including daily rainfall and temperature, host abundance and susceptibility, human population density, and pathogen dispersal characteristics. The parameterized model was implemented (1990 to 2005) in a GIS to simulate disease spread across California at a spatial resolution of 250 x 250 m. Model performance was also evaluated in the GIS by examining the spatial correspondence between predicted patterns of disease spread and 784 geo-located field observations of disease presence. The nature of prediction errors was examined by ecoregion, vegetation composition, and climate. The model predicts almost 80 percent of the spatial variability in current patterns of disease spread and identifies numerous forest ecosystems at high risk of infection. We believe the application of epidemiological models to realistic landscapes in a GIS can allow for a rigorous validation of model performance using geo-located field data of disease presence and can be used as an effective management tool to identify actual landscapes at high risk to disease spread.

Key words: *Phytophthora ramorum*, biological invasions, emerging infectious disease, spatial modeling, forest management, model validation, dispersal, GIS.

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Mapping Sudden Oak Death Risk Nationally Using Host, Climate, and Pathways Data[1]

Frank H. Koch[2] and William D. Smith[3]

Abstract

In 2002, a team of United States Department of Agriculture-Forest Service (USDA-FS) scientists developed a preliminary risk map to serve as the foundation for an efficient, cost-effective sample design for the national sudden oak death detection survey. At the time, a need to initiate rapid detection in the face of limited information on *Phytophthora ramorum* necessitated a simple spatial intersection of factors traditionally used in risk mapping: (1) distribution of hosts known or likely to be susceptible to the pathogen, (2) climatic conditions adequate for survival and propagation of the pathogen, and (3) pathways for introduction of the disease outside the currently infected region. The resulting map consisted of hexagons indicating three ordinal levels of risk (high, moderate, low) covering the conterminous U.S. Beyond aiding prioritization of sampling effort, the map has facilitated the dissemination of information about the potential threat of the disease to the public. However, additional information about the pathogen has emerged in the last few years, so we created a new map by incorporating current data and approaches to better depict principal factors of *P. ramorum* risk. The new risk map will be used to improve sampling procedures for detecting the pathogen's potential movement into wildland areas. In addition, it will serve as the basis for analysis of the potential economic costs if *P. ramorum* were to become established in eastern U.S. forests.

Key words: *Phytophthora ramorum*, sudden oak death, risk map, detection survey.

Introduction

By early 2002, *Phytophthora ramorum* had been detected on nursery stock in California and Europe, in addition to infesting forests in California and Oregon (COMTF 2007). This suggested the potential for *P. ramorum* to be dispersed long distances by infected plants. While the United States Department of Agriculture-Animal and Plant Health Inspection Service (APHIS) began efforts to regulate movement of nursery stock, concerns regarding the potential economic risk to the nation's oak forests led to the development of a national sudden oak death detection survey, managed by the USDA-FS. To facilitate this detection effort, a team of Forest Service researchers developed a risk map in 2002 that could serve as a national sampling frame. Their approach was based on three factors: 1) climatic conditions affecting the pathogen's distribution where it was established, or as determined from laboratory studies; 2) likely host species based on their distribution in areas where the pathogen was established, or as determined from laboratory inoculations; and 3) likely pathways of introduction into new areas (Smith and others 2002; U.S.

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Department of Agriculture Forest Service 2004). Areas at greatest risk for new introductions occurred where these three factors spatially coincided.

This preliminary risk map (fig. 1) has served its purpose of prioritizing sample placement, but after five years, more has become known about the pathogen in terms of basic epidemiology, suitable hosts, and suitable environmental conditions. Our primary objective was to create a new national risk map for *P. ramorum* incorporating current knowledge about the pathogen as well as additional data sources and techniques not utilized for the preliminary map. Recently, there have been several publications presenting national-scale maps related to *P. ramorum* risk. Venette and Cohen (2006) employed CLIMEX software to model suitability for establishment based on climatic variables, calculating parameter values from laboratory studies of *P. ramorum* and *P. cinnamomi*. Fowler and others (2006) similarly modeled infection risk using climatic variables and laboratory-based parameter estimates. Kluza and others (2007) modeled the ecological niche of *P. ramorum* from the known occurrences in Oregon and California, applying a genetic algorithm to topographic, climatic, and remotely sensed variables. Kelly and others (2007) compared the results of several different niche models developed from the known occurrences. These studies focused primarily on environmental constraints for *P. ramorum* and included, if at all, limited data on host species distribution. They also did not address potential pathways of introduction of the pathogen into forested landscapes. Our map is intended to provide a comprehensive depiction of risk that incorporates detailed host and pathways variables in addition to climatic constraints, thus making it useful for optimizing the national detection survey.

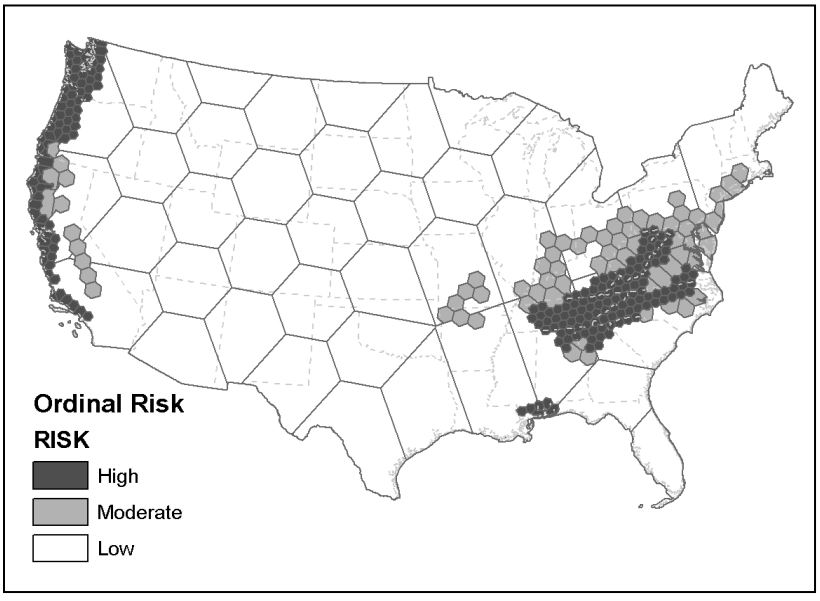

Figure 1—Preliminary national risk map (2002) for *P. ramorum.*

Methods

To construct a new map, we adopted a decision-rule-based approach with three basic steps. First, we assembled input data into three "surfaces" representing (1) host species distribution, (2) climatic suitability, and (3) potential pathways of *P. ramorum* introduction into natural forests. Second, we combined the host and climate surfaces into a surface that we have termed "hazard". In this context, hazard represents the relative likelihood of significant forest damage given a successful introduction. Third, we created a risk map composed of sampling hexagons in low, moderate and high risk size classes. We determined where to place the high and moderate risk hexagons based on threshold values for the hazard and introduction potential surfaces; in other words, the risk rating represents a combination of these two factors.

Host Surface

Only 14 forest species were known to be affected by *P. ramorum* in 2002. The new map's host surface incorporates the distribution of the several dozen species currently on the proven and associated host lists managed by APHIS (APHIS 2007). Another significant change from the 2002 map is the removal of certain oak (*Quercus*) species from the list of susceptible eastern U.S. hosts. Examination of infrageneric relationships between oak species revealed that western live oaks (including coast live oak, *Quercus agrifolia*) belong to the red oak group, while eastern live oaks (*Quercus virginiana* and other species) are members of the white oak group and thus apparently unsuitable hosts (Nixon 1993, 2002).

We constructed the host surface from three components: a background host layer, a midstory sporulators layer, and an overstory layer. The background host layer depicts areas of the U.S. with at least some hosts present, enabling propagation of *P. ramorum*. To build the layer, we used Floristic Synthesis of North America software (Kartesz 2007), which provides county-level distribution data for most plant species, both native and exotic, found in U.S. forests. For each county, we tallied occurrences of all non-oak host species on the APHIS lists into a total abundance score. When scoring, we assigned two species, California bay laurel (*Umbellularia californica*) and tanoak (*Lithocarpus densiflorus*), and one genus (*Rhododendron* spp.) multipliers of ten, five, and five, respectively, to reflect their importance to *P. ramorum* spread. To minimize the effect of any individual counties with incomplete records, we generalized the map at the ecoregion subsection level (McNab and others 2005).

California bay laurel and tanoak are common in forest midstories. Compared to other understory hosts, they can disperse *P. ramorum* spores over a larger area and may expose some mature hosts to potential infection. Based on preliminary data (Paul Tooley, unpublished data), we also identified four eastern U.S. forest species with high sporulation potential that commonly grow into the midstory: dogwood (*Cornus florida*), black locust (*Robinia pseudoacacia*), serviceberry (*Amelanchier canadensis*), and southern magnolia (*Magnolia grandiflora*). We used ordinary kriging to interpolate USDA-FS Forest Inventory and Analysis (FIA) Phase Two plot data into a national map depicting percentage of the total susceptible-species basal area composed of these midstory sporulators.

The overstory layer represents the distribution of hosts with potentially significant mortality due to *P. ramorum*. In addition to tanoak, this included all oak species from the true red and black oak group, the willow oak group, and the western live oak group, as well canyon live oak (*Q. chrysolepis*) from the intermediate oak group (Nixon 1993, 2002). We also included Pacific madrone (*Arbutus menziesii*) due to recent observation of mortality in the species due to *P. ramorum* (Ellen Goheen, personal communication). We used ordinary kriging to interpolate FIA plot data into a national grid of basal area.

We combined these three components into a single 1 km^2 resolution host surface by rescaling the background layer abundance scores and by evaluating where the midstory and overstory layers met thresholds of 10 percent and 3.44 m^2/ha (15 ft^2/ac) in basal area, respectively. The final surface had a four-point rating scale (table 1).

Table 1—Rating scale for final host surface

Rating	General Description
0	Oak hosts but no other host species
1	Limited (<3.44 m^2/ha basal area) oaks with some other hosts OR abundant (>=3.44 m^2/ha basal area) oaks with only non-sporulating hosts
2	Limited oaks with midstory sporulators comprising >10 percent of susceptible-species basal area OR abundant oaks with sporulating hosts, but no midstory sporulators
3	Abundant oaks, midstory sporulators, and additional sporulating hosts

Climate Surface

In the 2002 map, climatic limits for *P. ramorum* were defined using maps from the Climate Atlas of the United States. Climate Atlas maps are monthly or annual summaries, so they cannot capture the fine-temporal-scale, simultaneous occurrence of temperature and moisture conditions that may promote the pathogen's persistence (Garbelotto and others 2003, Rizzo and Garbelotto 2003). We used daily weather station data from the National Climatic Data Center (NCDC) to create annual grids (4 km^2 resolution) of the longest string of consecutive days where two conditions occurred simultaneously: (1) temperature between 15.56 and 26.67 °C during the day and (2) some precipitation, fog, or mist during the day OR mean relative humidity during the day of greater than 90 percent. We defined these conditions based on results of laboratory studies (Werres and others 2001, Rizzo and Garbelotto 2003, DEFRA 2004). We averaged 10 years (1997 to 2006) of annual grids into a single consecutive-day grid. We then divided the consecutive-day grid into two parts by ecoregion domain, placing the Humid Temperate and Humid Tropical Domains (Eastern U.S., Pacific Coast) in one super-region and the Dry Domain (Interior West, northern Great Plains) in another (McNab and others 2005). We reclassified the two super-regions using different rating scales (table 2)—accounting for factors such as the general moisture deficit in the Dry Domain (Bailey 1998)—and then recombined them into a single grid.

Table 2—Rating scales for climate variable, the largest number of consecutive days with co-occurrence of optimum temperatures and moisture, in two U.S. super-regions

Humid Super-region		Dry Super-region	
# Consecutive Days	Rating	# Consecutive Days	Rating
< 3.5	0	< 3.5	0
3.5 – 7	3	3.5 – 5	1
7 – 14	4	5 – 7	2
14 – 28	5	7 – 14	3
> 28	6	> 14	4

In the 2002 map, any areas with more than one winter month where the maximum temperature was below 0 °C or more than one summer where the maximum temperature was above 32.22 °C were considered unsuitable for long-term persistence of *P. ramorum* (David Rizzo, personal communication). For the new map, based on laboratory observation of infection after prolonged time periods at 0 °C (DEFRA 2004), we applied a cold temperature mask excluding areas where the minimum temperature was below 0 °C for 150 or more days. We did not use a high temperature mask because, as evidence from the Southern Appalachian region suggests (James Vose, unpublished data), areas under forest canopies may be as much as 3 to 4 °C cooler in the summer than non-forested areas, mitigating the deleterious effect of heat on pathogen survival.

Introduction Surface

In 2002, *P. ramorum* had been detected on nursery stock in a few instances, but the nursery industry had not yet experienced widespread impacts. The 2002 map did incorporate locations of rhododendron nurseries based on a national growers' association listing, but it has since been documented that infected nursery stock has been shipped from California, Oregon, and Washington to roughly 40 states in the past few years (Stokstad 2004), and that some of this stock was sold to customers before it could be destroyed. This emphasizes the possibility that *P. ramorum* could escape from ornamental plantings in residential landscapes into natural forests. To represent this possibility, we constructed an introduction surface from maps of wildland-urban interface. These maps, developed at the census block level, combine housing density information, land cover percentages, and forest proximity measurements to classify areas into one of thirteen classes according to the level of interspersion between residential areas and natural vegetation. We reclassified these maps for the entire U.S. to emphasize areas with moderate levels of intermix (table 3). We used a neighborhood function to define "edge" zones, areas where high-risk intermix and contiguous areas of natural vegetation are adjacent (table 3).

Table 3—Rating scale for introduction surface

Rating	Description
0	Excluded areas (highly urban, water, or natural vegetation not included in an edge zone)
1	Low risk (housing densities greater than 741.3 units/km^2)
2	Moderate risk (interface[a] with housing densities less than 741.3 units/km^2)
3	High risk (intermix[b] with housing densities less than 741.3 units/km^2)
4	Edge zone (high-risk intermix and natural vegetation immediately adjacent)

[a] natural vegetation occupies < 50 percent of area, but areas with > 75 percent natural vegetation are within 2.4 km

[b] natural vegetation occupies > 50 percent of area

Risk Map Construction

We created a national hazard surface by combining our host and climate surfaces using a simple equation: hazard score = host surface score * 10 + climate surface score. We then reclassified the result on a four-point scale (table 4). For the 2002 map, three hexagonal tessellations covering the conterminous U.S., with hexagon sizes increasing from low to high relative risk, were generated via intensification of the North American hexagon of the global Environmental Monitoring and Assessment (EMAP) sampling grid (White and others 1992); we used these same tessellations in the new map. To select which moderate and high risk hexagons were retained, we calculated mean hazard surface and mean introduction surface scores for each hexagon. For the high risk stratum, we retained any hexagon with a mean hazard score greater than 1.5 or a mean hazard score greater than 1.0 and a mean introduction score greater than 2.0. For the moderate risk stratum, we retained any hexagon with a mean hazard score greater than 1.0. In addition, we used the locations of positive nurseries from APHIS trace forwards to indicate potential introduction hotspots. These data served to promote hexagons in terms of risk; for example, if a hexagon had inadequate hazard or pathways scores to be considered high risk, but did contain one or more positive nurseries, it was retained in the high risk stratum. We overlaid the selected high and moderate risk hexagons on the background low risk hexagons to create our map.

Table 4—Rating scale for hazard surface

Rating	Hazard Score Range[a]	Description
0	n/a	Non-forested areas
1	0-21, 30	Poor climatic conditions and/or low host levels
2	22-26	Moderate host levels and adequate to good climatic conditions
3	33-36	High host levels and adequate to good climatic conditions

[a] Two possible scores from the hazard equation, 31 and 32, did not occur within the conterminous U.S.

Results and Discussion

A primary difference between the new risk map (fig. 2) and the 2002 map is an expansion of risk areas in the southeastern U.S., particularly in the Piedmont regions of North and South Carolina, Georgia, and Alabama. Coastal areas of Georgia, South Carolina, Alabama, and Mississippi have also been highlighted, as has northern Florida. Portions of the Mid-Atlantic region, along with parts of Tennessee,

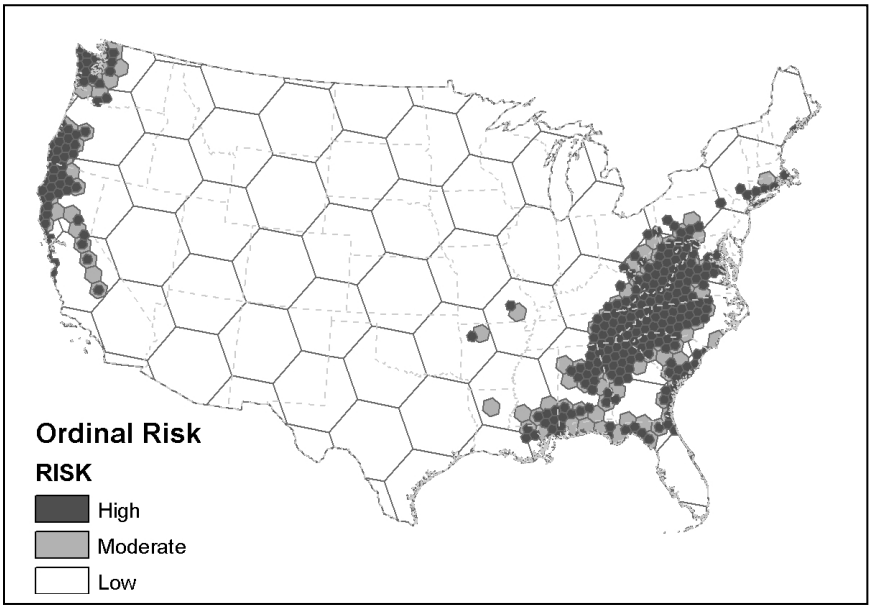

Figure 2—Revised national risk map for *P. ramorum.*

Kentucky, Indiana, and Ohio, shifted from moderate to low risk. Although we have not yet performed a planned sensitivity analysis, the appearance of moderate and high risk hexes throughout coastal areas of the Southeast appears to be largely linked to high host presence. Many of these areas were excluded by a high temperature mask in the 2002 map. How *P. ramorum* responds to extended periods of high temperatures remains an open question; as already noted, forest canopies may provide enough of a cooling effect to mitigate high summer temperatures (Geiger and others 2003). With respect to the Pacific Coast, our new map closely resembles the 2002 map, although the pattern of high and moderate risk hexes does not extend as far south along the California coast, basically ending south of the Big Sur region. Review of the input data layers suggests a lack of host species as the primary constraint. Part of this may be due to the scale and accuracy of available host data, but the extent of risk predicted in California and along the rest of the Pacific Coast appears consistent with results seen in other risk mapping efforts (for example, Meentemeyer and others 2004, Kelly and others 2007) that similarly included some representation of host species distribution.

With respect to the eastern U.S., our map is generally consistent with other studies (Venette and Cohen 2006, Kelly and others 2007, Kluza and others 2007) in locating the greatest risk in the Southeast. One major distinction is that our map ranks all of the Southern and Central Appalachian Mountain regions as high risk, while the other studies assigned lower risk ratings to at least part of these areas. This appears to be due in part to our emphasis on host dynamics, particularly the co-occurrence of critical understory species (for example, *Rhododendron* spp.) and susceptible oaks in the forested areas of these regions. However, high levels of wildland-urban intermix (in other words, introduction potential) in northern Georgia and elsewhere in the southern Appalachians contributed to the contiguity of high risk throughout the region.

We will perform sensitivity analysis to more precisely determine, for each moderate and high risk hexagon, which variables most contributed to their inclusion. We will also examine how differences from the 2002 map may have resulted from changes in methodology. After review and possible alteration, we hope to use the new map in the same fashion as the 2002 map to facilitate sampling procedures for detecting the pathogen outside its current extent. It will also serve as the basis for analysis of the potential economic costs if *P. ramorum* were to become established in eastern U.S. forests (see Holmes and Smith, this proceedings).

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Linking Sudden Oak Death With Spatial Economic Value Transfer[1]

Tom Holmes[2] and Bill Smith[2]

Abstract

Sudden oak death (caused by *Phytophthora ramorum*) is currently having a dramatic impact on the flow of ecosystem services provided by trees and forests in California. Timber species in California are not thought to be at risk of mortality from this pathogen and, consequently, economic impacts accrue to non-market values of trees such as aesthetics, shading, and the knowledge that healthy forest ecosystems exist. Because non-market valuation studies are expensive to design and implement, we propose that spatial benefit transfer methods can be used as a pragmatic means for obtaining second-best estimates of the economic damages associated with *P. ramorum*. Economic damages to residential property values and public forest land are identified as a major concern.

Key words: Economic impacts, non-market values, value transfer, hedonic property value, contingent valuation, existence value, GIS.

Introduction

Forests provide suitable habitat for an assortment of invading organisms (Liebhold and others 1995) and invasive species have been ranked as one of the four critical threats to our Nation's forest ecosystems by the Chief of the United States Department of Agriculture-Forest Service (USDA-FS) (USDA Forest Service 2004). Although most people might argue that it is laudable to counter threats to the structure and functioning of forest ecosystems, relatively few exotic organisms become a major pest (Williamson 1996). It is the main thesis of this paper that decisions regarding budget allocations and the targeting of forest protection efforts would benefit from a clear understanding of the costs and benefits of invasive forest pest management (Holmes and others 2007). Interventions designed to mitigate damages from exotic forest pests are costly - the USDA-FS spent $95.1 million dollars for the management of invasive forest pests in fiscal year 2005 (USDA Forest Service 2005, p. 14 to 55). However, very little is known about the magnitude of economic damages caused by exotic forest pests, or the efficacy of the money spent on pest control. This lack of knowledge impedes economic analyses of pest management programs and it remains unclear whether current expenditures on invasive forest pests are too little, too large, or about right.

Economic damages are incurred when invasive forest pests alter the flow of ecosystem services provided by trees and forests which are valued by people. Forest ecosystem services can be broadly categorized as services that are traded in markets (such as timber) and non-market-based services (such as aesthetically pleasing views). Market and non-market values can be evaluated using consumer and

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producer surplus concepts drawn from welfare economic theory. In instances where data are not available on the supply of, or demand for, economic goods and services, other non-welfare-theoretic methods may be adequate. Although it is often tempting to sum various economic measures to arrive at an aggregate estimate of damage, it is important not to sum economic measures that are based on different conceptual foundations. Likewise, it may not be meaningful to compare damage estimates computed using widely divergent economic methods.

Perhaps the most widely cited estimate of the aggregate damages from exotic forest pests ($4 billion per year) is due to Pimentel and others (2000). Their measure was computed by multiplying the price of timber by an estimate of the annual quantity of timber destroyed using pest loss rates estimated for Canadian forests. Their measure does not include the broader impacts of forest pests on non-market economic values and is therefore biased downwards, perhaps severely. For example, using such a methodology, the forest ecosystem disturbances occurring in California and Oregon from *P. ramorum* infections would not be causing any economic damage as important timber species in this region are not thought to be at risk of loss from this pathogen (Rizzo and others 2005). Other approaches are clearly required to estimate the economic damages associated with the loss of hundreds of thousands of trees in private and public landscapes currently being killed by this pathogen.

Spatial Benefit Transfer Methods

In this paper, we propose the use of benefit transfer methods for estimating the non-market value losses attributable to the spread of *P. ramorum* in California. The logic for using benefit transfer is pragmatic - non-market valuation studies are expensive to design and implement. When funds are unavailable for collecting primary data for a non-market valuation study, benefit transfer may provide a reasonable second-best alternative.

Broadly speaking, a benefit transfer uses estimates of economic value derived from research conducted at a study site and transfers the estimates to a policy site of interest. Various methods are available for conducting a benefit transfer study and which method is used depends upon the data available and the goals for conducting the transfer (Rosenberger and Loomis 2003). The *value transfer* approach directly applies summary statistics from the original research to the policy site. This approach would typically use a point estimate, some measure of central tendency, or an administratively approved estimate of value. The *function transfer* approach is more technically demanding than a simple *value transfer* as it involves the transfer of statistical models defining functional relationships between variables. Examples of this method would include the use of demand or willingness-to-pay (WTP) functions as well as meta-analysis regression functions. *Function transfers* may be more informative than *value transfers* as they can facilitate adjustments that account for salient characteristics at the policy site. Both the value and function transfer approaches require the acquisition of data from existing studies which may be identified in published and unpublished literatures.

The damage caused by invasive species is intrinsically spatial in nature and identifying the spatial incidence of damages improves the calibration of benefit transfer estimates, especially when damages occur over broad geographic areas and

land uses. Although most benefit transfer studies have been non-spatial, the increasing prominence of Geographic Information System (GIS) technology is advancing the ability to conduct spatial value transfers. Eade and Moran (1996) provide an early example of spatial value transfer by mapping the quality of natural capital assets in Rio Branco, Belize and then re-calibrating benefit transfer estimates using the quality maps. Bateman and others (2002) describe how travel cost assessments of recreational value can be transferred using the analytic capabilities of a GIS. More recently, Troy and Wilson (2006) developed a typology of land cover and aquatic resources which were spatially linked with economic valuation studies in three study sites.

At a landscape scale, trees and forests provide a heterogeneous array of ecosystem services. Humans recognize and value some sub-set of this array. A linkage between ecosystem services and economic value is provided by the recognition that human land uses reflect decisions about the provision of goods and services valued by people. For example, people choose to live in certain areas of the overall landscape, work in other areas, and set aside special locations for public use or scientific study. Within each of these land uses, forest ecosystem goods and services (such as trees) are managed and valued differently. Thus, one method of linking the flow of forest ecosystem services with economic values across a landscape is to identify the relationship between ecosystem characteristics, land-uses, and economic value.

Recognizing that economic values are not absolute, but rather represent the value of changes from a starting point or *status quo* state to an alternate state, the approach we recommend in this paper is to evaluate how changes in forest ecosystem characteristics alter the value of non-market good and service flows within separable land uses. In particular, the spatial benefit transfer method we propose is based upon a simple algorithm for identifying linkages between land uses, forest disturbances caused by *P. ramorum* and non-market economic damages:

$$Economic\,damage_i = [(Area_i) \cap (SOD)] * (value\Delta_i) \quad \forall i \tag{1}$$

where $Area_i$ is the area in land use type i, SOD is the area of *P. ramorum* infestation $value\Delta_i$ is the value change in land use type i resulting from *P. ramorum* infection, and $\cap$ is the intersection operator. It is important to note in equation (1) that economic impacts are computed separately for each land use category and yet are not summed across land uses. This is because economic estimates of value changes in forest health provided by the economics literatures are partial (rather than general) equilibrium impacts. Because the degree of interaction between forest health value on different classes of land use are unknown, summing the values across land use classes would lead to a biased estimate of aggregate impact unless the degree of interaction between land use classes is known to be zero. As we are unaware of any studies estimating the linkages between forest health values across land uses, we recommend evaluating each land-use class separately. None-the-less, categorical estimates can provide an indication of the relative importance of changes in forest health on various land use classes of interest as long as estimates are based on the same theoretical foundation.

Data Requirements

Three types of data are required to implement the economic value transfer method as shown in the above equation: (1) economic value estimates drawn from studies conducted at original study sites, (2) GIS data on forest health conditions in the policy area, (3) GIS data on land uses in the policy area. Each data requirement will be discussed in turn.

Economic Value Transfer Data

The economic literature on the non-market economic value of trees and forests has been focused primarily on the value of trees in residential and public landscapes. Consequently, the studies we briefly review are thought to be representative of the non-market economic values that trees add to urban and public forests.

Data Assessed With the Hedonic Property Value Method

One method of evaluating the economic impacts of invasive species on residential properties is to use econometric methods to tease out the change in property value due to a change in tree numbers or a decline in tree health while controlling for all other factors influencing housing prices. This method, known as the hedonic property value method (HPM), has the advantage of utilizing data on actual economic transactions. Several economic studies have been conducted using (HPM) to estimate the contribution that trees make to private property values in residential landscapes (table 1). An early study was provided by Morales and others (1976) who found that tree cover added about 6 percent to property values in Manchester, Connecticut. A similar result was reported by Anderson and Cordell (1988) who concluded that trees in the front yard of homes in Athens, Georgia added about 3 to 5 percent to housing prices relative to houses without trees (and each large front yard tree added about 0.88 percent to property value.)

Payne and others (1973) estimated that, on average, each large tree (> 6 in. [15.24 cm] dbh) added about $270 to property value in Amherst, Massachusetts. They also reported that the value arc elasticity (ε) for trees was 0.24. This means that a 1 percent increase (decrease) in the number of trees increases (decreases) property value by 0.24 percent. A somewhat larger arc elasticity ($\varepsilon = 0.66$) was found by Holmes and others (2006) for healthy hemlock trees on properties in Sparta, New Jersey.

In general, the available economic literature indicates that trees add, on average, from 1 to 5 percent to the value of private property in a residential setting. This stylized fact is consistent with Dombrow and others (2000) who, using tree appraisal methods, found that mature trees contributed about 2 percent to the value of single-family homes in Baton Rouge, Louisiana. Note that these values provide an estimate of the value of trees as a "stock" or asset value which, in turn, provides a flow of services over time. An estimate of the "flow" (for example, annual) value of residential trees would need to adjust these estimates for the length of time over which trees provide this service.

If trees make a positive contribution to property values then, presumably, factors that cause the demise of trees will reduce property values. However, it is not obvious that the loss in value due to either a loss in tree health or numbers will be symmetric with

an equivalent gain in health or numbers. Consequently, measures of the loss in property value due to a loss of tree health would improve the precision of economic damage estimates. We are only aware of two studies that directly estimate the loss in private property values from a decline in forest health. Holmes and others (2006) studied the impact of an exotic forest insect, the hemlock woolly adelgid, on property values in Sparta, New Jersey (table 1). They found that hemlocks with declining health decreased property values both for the property owner as well as having spill-over impacts on other properties in the neighborhood. Notably the absolute value of the (negative) value arc elasticity ($\varepsilon = 0.96$) for hemlocks in declining health was greater than the arc elasticity (positive) for healthy hemlocks ($\varepsilon = 0.66$). In a related study, conducted with a richer dataset in West Milford, New Jersey, Huggett and others (2007) found that property values decreased substantially during the later stages of hemlock woolly adelgid infestations when most hemlocks were either dead or dying. Their estimates show that for each 1 acre (0.4 ha) increase in dead and dying hemlocks, property values were reduced by about 8 percent in this housing market. Further, this study indicates that the major economic impact of a change in forest health occurs when most of the host trees are dead or dying. Thus, the time dimension is critical when estimating economic impacts.

Thompson and others (1999) used the hedonic pricing method to evaluate the change in private property value in Lake Tahoe, California resulting from tree mortality due to native insect infestations. In particular, their methodology estimated the change in property value due to thinning and removal of dead and dying trees. They identified substantial gains in value (1 to 30 percent) which were attributed to both improved visibility and a reduction of fire hazard.

Data Assessed With the Contingent Valuation Method

A second method for estimating the economic value of changes in forest health is to use surveys to elicit stated values from a random sample of the population. This method, known as the contingent valuation method (CVM), has the advantage that it is the only method capable of eliciting existence values, or the value of knowing a resource (such as a healthy forest) exists, even if one never anticipates visiting that resource. Although the CVM is typically used to estimate the non-market economic value of public resources, Walsh and others (1981a, 1981b) used this method to estimate the reduction in private property values in the Rocky Mountains of Colorado due to infestations of native forest insects. Contingent valuation surveys were implemented via interviews both with private property owners and with real estate appraisers. Estimates of value arc elasticity were similar across groups and indicated that a 1 percent increase in the area of visible damage from native forest pests results in 2.3 to 2.5 percent reduction in property value.

The CVM has also been used to assess the economic impacts of changes in forest health on public forest land. Several analyses have been conducted using contingent valuation data that were collected to evaluate public WTP to protect high elevation spruce-fir forests in the southern Appalachian Mountains from the exotic balsam woolly adelgid (BWA). These analyses (Haefele and others1992, Holmes and Kramer 1995, Holmes and Kramer 1996, Kramer and others 2003) indicated that: (1) median annual household WTP for BWA protection programs ranges from $28 to $36 and, (2) existence value is an important component of total forest value.

Table 1—Sources for non-market economic valuation of trees and forests

Value type & location	Annual benefit/ loss	Economic unit	Economic method	Data source
Private property value				
Healthy trees				
Connecticut	6 percent house value	Urban forest tree numbers	HPM	Morales and others (1976)
Georgia	3 to 5 percent house value (0.88 percent per large front yard tree)	Urban forest tree numbers	HPM	Anderson & Cordell (1988)
Massachusetts	ε = 0.24 house value	Urban forest tree numbers	HPM	Payne and others (1973)
New Jersey	ε = 0.66 house value	Ex-urban forest tree area	HPM	Holmes and others (2006)
Louisiana	2 percent house value	Urban forest tree numbers	Tree appraisal	Dombrow and others (2000)
Dead and dying trees				
New Jersey	ε = 0.96 house value	Ex-urban tree area	HPM	Holmes and others (2006)
New Jersey	8 percent house value (per ac.)	Ex-urban tree area	HPM	Huggett and others (in press)
Colorado	ε = 2.27 house value	Rural visible damage	CVM – owners	Walsh and others (1981a)
Colorado	ε = 2.48 house value	Rural visible damage	CVM – appraisers	Walsh and others (1981b)
California	1 to 30 percent house value	Thin unhealthy trees in resort area	HPM	Thompson and others (1999)
Public forest value				
Healthy trees				
California	$477/ tree	Urban forest tree numbers	CTLA	Nowak and others (2002)
California	$27.12/ resident $54.33/ tree	Urban forest tree numbers	Various	McPherson and others (1999)
Dead and dying trees				
North Carolina	Median WTP = $28 to $36/ hhd.	National Forest/ Park area	CVM – households	Holmes and Kramer (1996); Kramer and others (2003)
Colorado	Mean WTP = $52/ hhd.	National Forest area	CVM - households	Walsh and others (1990)

Note: HPM refers to the hedonic price method, CVM refers to the contingent valuation method, CTLA refers to the Council of Tree and Landscape Appraisers.

In a similar study, Walsh and others (1990) used the contingent valuation method to estimate public WTP to protect national forests in Colorado from native insect infestations. Their value estimate (average annual household WTP = $52) was similar to the values reported for the southern Appalachian Mountains. In addition, they reported that non-use values dominated the estimates of use value. Taken together, these studies indicate that the public holds a significant willingness to pay for forest health protection on public lands, and that non-use values are an important component of total economic value.

It should be pointed out that the contingent valuation method is not without its critics, and that criticism stems largely from the fact that WTP estimates are based on stated

intentions rather than actual expenditures. Further, forest health protection studies conducted using the contingent valuation method have not fully incorporated the dynamic nature of forest disturbances. That is, forest succession will follow some (perhaps largely unknown) pathway following mortality due to either native or exotic forest pests. Thus, WTP estimates need to take this dynamic into consideration. Finally, because WTP estimates are typically estimated for households, an estimate of aggregate impact requires that the "extent of the market", or the number of households holding a positive WTP for the public forest at risk, be determined.

Data Assessed With Accounting Methods

Finally, it should be recognized that a few studies have been published that seek to estimate the value of trees in the urban forest using accounting methods. These studies are conducted using either a tree appraisal approach (for example, Nowak and others 2002) or mixed methods (for example, McPherson and others 1999). Tree appraisal methods use a formula to estimate tree value based on the physical characteristics of a tree. Although tree appraisal methods are not based on theoretic economic models, they appear to provide fairly similar estimates to the hedonic pricing method when applied to residential properties. The application of tree appraisal methods to other land uses, such as the value of street trees, cannot be similarly compared to theoretic economic models because we are unaware of such studies. Other studies that use mixed methods for valuation estimate an aggregate value of trees in the urban forest by summing values for the various services that trees provide, such as aesthetics, shading, and moderating water runoff. As noted above, the summation of values estimated using dissimilar methods (such as hedonic property values and estimates of energy savings from the cooling function of trees in summer) is not consistent with standard economic theory.

Forest Health and Land Use GIS Data

A review of the non-market economic value studies above provides us with an indication of the types of forest cover and land use data that would be required to conduct an economic value transfer study. To match up with the economic value data, GIS forest health data would need to be available for the policy site(s) that include the spatial location and area, number, or percentage of trees that are hosts for the pathogen, metrics concerning the area, number, or percentage of trees that are currently infected, and the anticipated rate of spread. In addition, as the economic literature suggests that economic impacts are most pronounced during the period of time when host trees are dead or dying, spatially reference data would be required for the timing and location of tree mortality induced by *P. ramorum* dispersal.

The land use data required for economic value transfer would include GIS data depicting the location and area of tree hosts on land uses such as urban residential properties (for transfer of HPM values) and public lands such as local, state, and national parks and forests with tree species that are at risk of mortality from *P. ramorum* (for transfer of CVM values). Remote sensing data have proven to be successful in mapping *P. ramorum* incidence in California at fine spatial resolutions (Kelly and Meetenmeyer 2002).

An example of the type of GIS data required for economic value transfer are GIS data showing the location and perimeters of concentrated confirmed areas of *P. ramorum* infected vegetation which were first published in January, 2002 by the sudden oak

death project, Center for Assessment and Monitoring of Forest Environment Resources (CAMFER), University of California Berkeley (figure 1). Data on land use classes are also available on the same website. The data show 22 distinct areas of *P. ramorum* infestation within Marin County, California which range in size from 46.2 to 10,126 ha, and total approximately 30,568 ha countywide. Of this total, approximately 15,002 ha fall within local, state, and federal park land and 11,349 ha fall within the urban residential land use class (greater than 1 housing unit per acre). Estimates of the average annual number, area, or percentage of trees that die from *P. ramorum* infections would allow estimates of non-market economic losses to be computed. A good example is provided by Kelly and Meetenmeyer (2002) who estimated that about six dead and dying trees per hectare could be identified in and around China Camp State Park in Marin County, California.

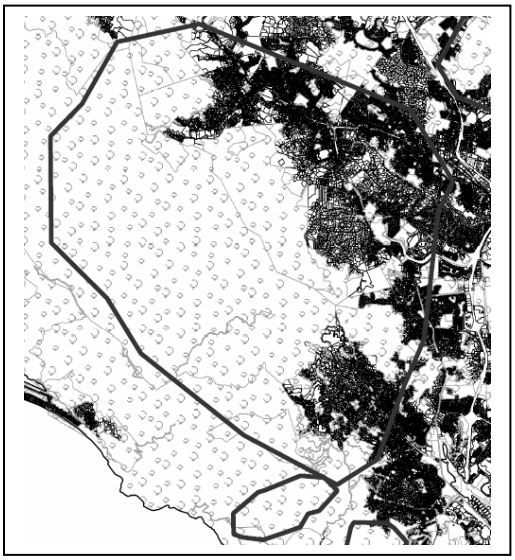

Figure 1—GIS map showing concentrated confirmed areas of *P. ramorum* in the San Raphael – Novato community, Marin County, California. The approximate perimeters of infested areas are shown as grey polygons. Darkly shaded areas are urban parcels, and the lightly shaded areas are public parks.

Conclusion

This paper argues that economic value transfer methodology can be used to provide second-best estimates of damages to non-market economic values associated with changes in forest health due to *P. ramorum* infections in California. This research program requires data on non-market values provided by the literature in combination with spatially reference GIS data showing the location of trees currently infected with the pathogen, regions at risk of future dispersal of the pathogen, anticipated rates of dispersal, and estimates of mortality rates. Although it is thought that the value transfer methodology could be used to evaluate the non-market economic impacts of *P. ramorum* throughout California, it is recommended that a preliminary evaluation of this method be conducted by completing a spatially explicit economic value transfer in one or a few counties of the state that are currently experiencing severe impacts from this exotic forest pathogen.

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Management

Lithocarpus densiflorus

Vegetation Response Following *Phytophthora ramorum* Eradication Treatments in Southwest Oregon Forests[1]

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

Abstract

Sudden oak death, caused by *Phytophthora ramorum,* was identified in late July 2001 in forest stands in Curry County on the southwest Oregon coast where it was killing tanoak (*Lithocarpus densiflorus*) and infecting Pacific rhododendron (*Rhododendron macrophyllum*) and evergreen huckleberry (*Vaccinium ovatum*). Affected stands occurred on industrial forest land, non-industrial forest land, and federal forest land. Treatments to eradicate the pathogen from affected sites were started in the fall of 2001 and consisted at that time of cutting, piling, and burning all infected host vegetation and any known Oregon host species within a 15 to 30 m buffer around all infected plants. While a number of plant species on the official host or associated host lists occurred in Oregon forests, only those plant species that had a history of being infected in Oregon were treated. Patch size of the initial treatment areas ranged from 0.2 to 4.5 ha. Since that time, additional disease centers have been identified and eradication treatments have been completed at every site. Some treatments were adjacent to sites treated previously while others involved distinct new centers. Size of treated sites has varied widely. Over the last 5 years, treatment methods have been altered to reflect increased understanding of host susceptibility and pathogen survival and spread. Additional treatment components have included various combinations, where possible, of backpack herbicide spraying to kill stump sprouts, stump-top treatments with herbicides to prevent tanoak sprouting, injecting all tanoaks greater than 2.5 cm diameter with herbicides to prevent sprouting, raking, piling, and burning all Oregon host material, and increasing buffer width to 100 m. Some sites have been planted with conifer seedlings while others have not. The end result is a mosaic of treatment patch sizes, shapes, and structures including sites where 1) Douglas-fir (*Pseudotsuga menziesii*) logs were removed from the sites and the tanoaks and understory species were cut and destroyed in broadcast burns, 2) sites where 10 to 20 percent of the conifer overstory remains and the treatment area has been replanted with conifer seedlings, 3) a site where little change has occurred in an old-growth coast redwood (*Sequoia sempervirens*)/Douglas-fir overstory but more than 90 percent of the midstory and understory trees and shrubs were removed, and 4) small (less than 0.2 ha) openings with non-host mature alder trees left on site.

Most affected stands in southwest Oregon occur in the Douglas-fir/Tanoak forest type and have an original overstory component comprised predominantly of tanoak and Douglas-fir, with lesser amounts of red alder (*Alnus rubra*), Oregon myrtle (*Umbellularia californica*), cascara (*Frangula purshiana*), Pacific madrone (*Arbutus menziesii*), and bigleaf maple (*Acer macrophyllum*). In 2006, an infested site in a Coast Redwood/Tanoak forest type was

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confirmed and subsequently treated. On that site, Douglas-fir and coast redwood make up the overstory and tanoak was the predominant component of the midstory prior to treatment. The understory species mix in both forest types treated includes substantial amounts of tanoak, Pacific rhododendron, Oregon myrtle, and evergreen huckleberry, as well as a number of other shrub and herb species. Many of the species in both the overstory and understory produce prolific stump sprouts following cutting or burning.

Treated sites are being monitored for the presence of the pathogen using a variety of techniques including stream-water baiting with tanoak and rhododendron leaves, soil sampling and baiting using pear and leaf baits, and collecting vegetation samples on site and subjecting them to isolation and PCR-based diagnostic tests. While a few of the sites treated initially are now evidently free of the pathogen, on many sites *P. ramorum*, is still present in the soil or can be baited from streams flowing through treatment areas.

There is also a strong interest in monitoring both planted and natural vegetation on these sites. This interest stems from the desire to quantify and qualify how vegetation responds to treatment protocols, how vegetation responds in the presence of the pathogen, and whether the resulting vegetation structure and composition on the sites following treatments is consistent with overall landowner objectives.

A series of variable-radius and fixed area plots designed to sample between 5.0 to 7.5 percent of the treated areas were established to describe vegetation condition before and after eradication on a selection of sites. Trees greater than 13 cm dbh were accounted for in variable-radius plots. Trees less than 13 cm dbh were tallied and percent cover of shrub species was estimated on 0.02 ha circular plots. In some cases, pre-eradication conditions were determined from plots just outside treated areas but in similar forest types. Plants on plots and on transects between plots were examined for symptoms of *P. ramorum* infection. ELISA tests using field diagnostic kits were used to prescreen some symptomatic tissues for *Phytophthora* species infection. Most suspicious samples were sent to the laboratory at Oregon State University for identification.

Vegetation data 5 years after treatment show little change in overall species composition on treated sites, with the obvious exceptions that the overstory tanoak component on treated sites was eliminated in all cases, and on two sites, conifers not present before treatment were planted after treatment. In situations other than industrial forest lands where merchantable Douglas-firs within treated sites were cut and logs were removed to a nearby mill, conifers such as Douglas-fir or coast redwood, or non-host hardwoods such as red alder were left standing on sites. There were substantial changes in the percent cover occupied by understory tanoak, evergreen huckleberry, and Pacific rhododendron after treatment; however, these species were not entirely eliminated on any treated site. Tanoak sprouts were sometimes missed in post-treatment herbicide sprays. Evergreen huckleberry and Pacific rhododendron were not routinely subjected to herbicides and many sprouted back after cutting. Percent cover of sword fern (*Polystichum munitum*) generally increased after treatments. No samples collected on plots or along transects between plots were found to be infected by *P. ramorum*.

Early eradication prescriptions involved only the cutting and burning of infected and buffer host plants. When resprouting tanoaks and evergreen huckleberry plants on several sites were confirmed to be infected, herbicide treatment to kill stump sprouts was immediately done. In 2003, 3 m-diameter plots on five of the treatment areas were located around 43 stumps resulting from the cutting of known infected trees. Douglas-fir and redwood seedlings were deliberately planted as *P. ramorum* susceptible baits around each of the stumps on some sites while on others, seedlings planted as a part of post-eradication reforestation were used as bait. Sprouting tanoak, Pacific rhododendron, and evergreen huckleberry were also monitored for infection. These stump-based plots have been revisited and vegetation condition of all host plants on each plot has been reassessed several times since the initial plot installation, with the

most recent evaluation done in January 2007. Since the initial identification of infected tanoak and evergreen huckleberry sprouts, no vegetation on these plots, planted or natural, has been found infected.

In May 2003 soil samples were collected at the base of the selected stumps and baited for the presence of *P. ramorum*. Soil sampling at these stumps has continued through the present on most sites. *Phytophthora ramorum* has been recovered from soil samples periodically since 2003 at the base of selected stumps on three of the sites, was recovered only in the initial sampling on one site, and was never recovered from soil samples on one site (table 1)

Table 1—Number of samples where *Phytophthora ramorum* was baited from soil collected adjacent to stumps of previously infected trees at five eradication treatment sites in Curry County, Oregon

Site	Sample date				
	5/2003	7/2004	12/2004	6/2005	1/2007
10	2/8*	0/8	0/7	0/8	0/8
11	0/5	0/5	0/1	1/4	0/3
18	1/8	0/8	1/8	3/8	2/8
33	3/17	0/5	7/16	4/17	8/13
36	0/5	0/5	Not sampled	0/5	Not sampled

* Number of stumps where *P. ramorum* was recovered from adjacent soil/Total number of stumps sampled at the site

Conclusions from monitoring vegetation response to *P. ramorum* eradication treatments in southwest Oregon 5 years after treatment include the following:

- Most components of the native flora found in Douglas-fir/Tanoak and Coast Redwood/Tanoak ecosystems are essentially intact on eradication sites after treatment, excepting tanoak which has been removed from the overstory and midstory of all treated sites.
- Openings create conditions apparently unfavorable for *P. ramorum*. No new infections have been found on host species regenerating or surviving within treated areas. This includes host species in close proximity to known infested stumps or where soil remains infested.
- Landowner objectives for growing conifer species are not compromised. Planted Douglas-fir and coast redwood seedlings/saplings associated with known infested stumps and in areas where soil remains infested are healthy.
- The structural component of habitat for northern spotted owl in the Coast Redwood/Tanoak site has been temporarily altered due to reductions in the midstory and understory; however, according to local wildlife biologists, conditions appear to be good for foraging.

Vegetation monitoring on treated sites will continue.

Key words: *Phytophthora ramorum*, sudden oak death, eradication.

Wildland Management of *Phytophthora ramorum* in Northern California Forests[1]

Yana Valachovic,[2] Chris Lee,[3] Jack Marshall,[4] and Hugh Scanlon[5]

Abstract

In early 2006 we implemented a series of comparative silvicultural treatments aimed at managing the spread of *Phytophthora ramorum* Werres, de Cock & Man in't Veld by reducing inoculum densities in isolated infestations on one public and three private properties in southern Humboldt County. These treatments, which took place on over 56 forested ha, are the first attempts at large-scale, stand-level silvicultural management of *P. ramorum* in California. As part of first-year post treatment monitoring, we compare a number of issues related to operational effectiveness of the treatment installations, such as identifying areas for treatment, barriers to treatment, timeframes, expense, equipment and vehicle sanitation, and public perception of treatments. These are issues to be considered by anyone attempting silvicultural treatment of this pathogen in the future.

Key words: *Phytophthora ramorum*, California, forest, management, silviculture.

Introduction

Wildland management of *Phytophthora ramorum* in California forests has generally been limited to a few small-scale projects. To date, the efforts have focused on preventing pathogen establishment by the use of phosphonate fungicide on individual oak trees (Garbelotto and others 2003) or the use of prescribed fire to remove understory hosts (Kent Julin, personal communication, 2006). By comparison, a larger-scale eradication program has been implemented in Oregon that involves use of herbicides (to prevent sprouting), host tree removal (to remove inoculum and pathogen reproduction), and pile burning infected materials (to sanitize the site) (Goheen and others 2004).

Phytophthora ramorum is currently established in a range of coastal California forest types, and the tools for managing the pathogen are not equally applicable to all of these forest types. Furthermore, the social acceptability of these tools is a critical component of implementation success. In forest areas already infested with the

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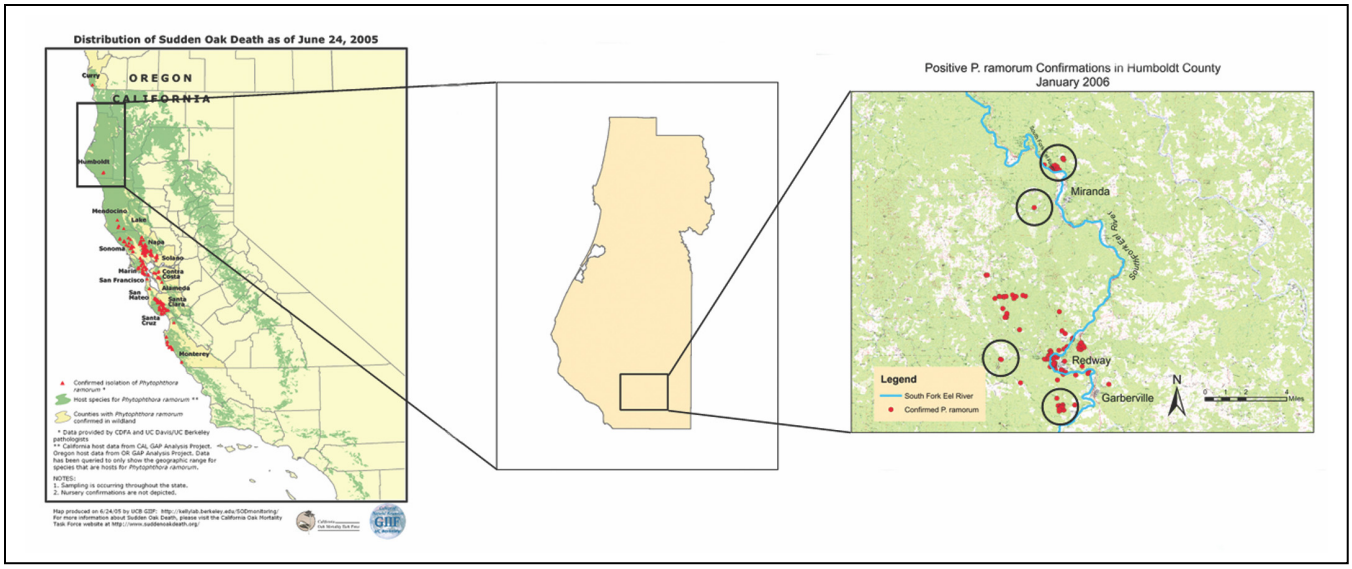
disease, more information is needed to better understand if it is possible to suppress inoculum production or slow the spread of the disease and to develop a range of long-term management recommendations for landowners and managers of forested properties. The goal of the study is to find treatment options that are socially acceptable, financially affordable and biologically effective in controlling the pathogen.

Materials and Methods

In Humboldt County, California, *P. ramorum* has been limited to one geographic region, in the South Fork Eel River watershed, presumably emanating from the area of the first report of the organism in 2002 in the understory of an old-growth redwood (*Sequoia sempervirens* [D. Don] Endl.) forest adjacent to the small town of Redway. Following an initial control effort in 2004 that involved removal of infected California bay laurel (*Umbellularia californica* [Hook. & Arn.] Nutt.) trees, the only known host at the time in the area, interest in managing the pathogen in this area geographically isolated from the rest of the *P. ramorum* distribution in California has been increasing. Since this initial find in the Redway area, a group has formed to implement an aggressive early detection program utilizing stream baiting, aerial reconnaissance, public education and targeted ground surveys.

Using information about areas infested with the pathogen, we developed a control strategy that prioritized installation of treatments to areas that were small (<20 ha) and on the outside of the core infested area around Redway. We reasoned that these areas represented advancement of the disease front within the county. The strategy envisaged a long-term disease suppression campaign resembling the effort involved in large-scale fire suppression, in which personnel attack "hot spots" that flare up outside the main body of the fire while performing a holding action on the main body.

We identified four areas (fig. 1) for treatment as a part of this adaptive management strategy in November 2005. These four areas comprised three privately owned



Figure—1. *P. ramorum* distribution in California and Humboldt County, and areas prioritized for treatment.

parcels and one publicly owned parcel. Prior to treatment, we established permanent monitoring plots for pre- and post-treatment data collection (circular, 0.04 ha in size, uniformly distributed in a 100 m X 100 m grid over each property) to provide an approximately 5 percent area sample. On these plots, we collected data characterizing vegetation conditions at the stand level (for example plant species present; numbers of and presence of symptoms on trees, saplings and seedlings; tree diameters; overstory canopy closure; basal area). Treatment implementation took place from January through May 2006.

The overall treatment program involved removal of the two main hosts involved in sporulation and aerial transmission of the pathogen, California bay laurel and tanoak (*Lithocarpus densiflorus* [Hook. & Arn.] Rehd.) (Rizzo and others 2005). This removal resulted in changes in stand structure designed to reduce humidity levels and understory host material. The specific treatments (table 1) included manual removal by chainsaw and pile burning of branches and foliage of all bay and tanoak, with and without subsequent underburning, replicated on three properties in October 2006. Additional, unreplicated smaller case-study treatments on the properties involved the removal of bay and tanoak using herbicides and thinning according to a standard fuels reduction prescription modified to include removal of all bay trees. These treatments were tested in two different but common forest vegetation types in northern California (table 1).

Table 1—Comparison of specific treatments, goals, effectiveness, and costs, one year after treatment

Treatment[6]	Goals	Effectiveness	Costs Public Lands	Costs Private Crews
bay + tanoak[1 2]	Remove abundant inoculum	Numerous saplings and seedlings left	$617.50/ ha	$4940-8645/ ha
bay + tanoak + broadcast fire[1 2]	Remove abundant inoculum, fire to consume seedlings, saplings, litter	Fire consumed saplings, seedlings, litter	$988/ ha	$4940-8645/ ha
herbicide bay and tanoak[2]	Remove abundant inoculum + prevent sprouting	Tanoak died in 3 months, Bay alive 14 post treatment	NA	$617.50/ ha
fuel hazard reduction + bay[2]	Test common treatment, girdle large diameter bays	Residual tanoak alive, except near girdled bays that are still alive 10 months post treatment	NA	$4940-6175/ ha

[6] Comparison of forest stand conditions (Sawyer and Keeler-Wolf 1995) where treatments were tested, in superscript.
[1] Redwood alliance with some co-dominant Douglas-fir and a secondary story of mixed evergreen hardwoods;
[2] Douglas-fir-tanoak alliance with a secondary story of mixed evergreen hardwoods. The first, second, and fourth treatments listed in the table involved manual removal of tree species accompanied by hand piling and burning of branches and foliage. The herbicide treatments did not involve processing of branches or foliage.

Given that it can be very costly to remove large bay trees and process their abundant foliage and that the trees sprout from the root collar after being cut, we tested two alternative treatment methods: the use of herbicides to kill trees in place and to prevent future sprouting, and girdling in place. The herbicide treatments involved a syringe-based injection of imazapyr in February 2006 (the earliest we could treat after finding the disease on the property in November 2005), since direct application via injection has been found to be the most effective method of imazapyr delivery for control of tanoak (DiTomaso and others 2004) and is much less costly than making future entries to control sprouts. Applicators spaced hatchet frills for herbicide injection every 7.5 cm on tanoaks and every 2.5 cm on bays, with 1 mL of chemical injected into each frill. Two forms of imazapyr were used, Arsenal® on upland trees and Habitat®, an aquatic formulation of the same chemical, for trees in riparian areas. To girdle large bays, crews cut into the trees with chainsaws to a depth of 15 cm around each entire tree in two locations approximately 0.3 to 0.6 m apart and used axes to completely remove all cambium and phloem tissue between the two cuts. The bay trees were girdled in May 2006.

We will ascertain pathogen persistence by assessing disease symptoms on new sprouts and seedlings of host trees each year for at least two years after treatment. The current assessment of these adaptive silvicultural projects reflects the state of our knowledge one year after treatment. So far we have conducted one-year post-treatment monitoring on one treatment area. More data will become available over time.

Results and Discussion

Approximately 1 year has lapsed since the beginning of treatment on these project areas. At this time we know some information about the effectiveness of the treatments, but more about the costs and challenges of implementing them.

Challenges for Implementation

A first challenge is that silvicultural management of bay and tanoak must take account of these species' ability to sprout following tree removal. By the end of the summer 2006 the cut bay and tanoak trees had sprouted in all treatment sites, in some cases reaching heights over 2 m tall. To our surprise, most bay logs left on the treated areas had also sprouted, although by the end of the summer, they had lost their vigor and had begun to die. We conducted 1-year post-treatment monitoring on one treatment area in February 2007. In one of the largest sites treated, the crews had a limited time window for operation (see below), and as we expected the crews were not able to remove all of the small diameter (<2 cm) host material. We found that the broadcast burn was an effective tool for removing this small-diameter material. In the unburned sites with more small-diameter host stems left, we do not know whether these stems will significantly contribute to aerial dispersal and spread of the pathogen, though we will continue to monitor these sites for new symptom development. Our one-year post-treatment assessment in one of the four treated areas (mentioned above) has so far found that new sprouts of tanoak and bay do not show signs of the pathogen. Kanaskie and others (2005) found that new tanoak stump sprouts could become infected following treatment. At this point we believe that it is too early in the season to draw conclusions about new infections of sprouts and seedlings. It may take several years to evaluate effectiveness of these treatments.

Initial management projects of disease infestations require the determination of specific boundaries of the infested areas. This is particularly challenging in the case of *P. ramorum.* In all cases the infested areas were a part of the larger forest, and there were no clear ecotones that would suggest logical boundaries for treatment areas. As part of our strategy to define the area for treatment, we carefully surveyed the areas surrounding infected trees for symptoms, primarily on bay leaves, and sent symptomatic material to the University of California Davis Rizzo lab for diagnosis. Once we were able to map general locations of the pathogen with the results from the laboratory testing, we conducted detailed field work to find exact perimeters where symptoms stopped. As noted by others (John Bienapfl, personal communication, 2005), it is particularly difficult to use symptoms on tanoak as an indicator of *P. ramorum* infection; where bay is present, symptomatic bay leaves serve as the best indicator of likely *P. ramorum* infection, even though several other pathogens cause similar symptoms.

Another consideration is timing of treatment operations, which in our studies was limited by both wildlife restrictions and rapid disease spread. In three of the four treatment areas, we lacked sensitive wildlife species data and so had to abide by all nesting restrictions for wildlife that could potentially occupy these sites. One of the treatment areas was determined to be potential habitat for the marbled murrelet, *Brachyramphus marmoratus* (Gmelin, 1789); this required that noise-producing (for example, chainsaw) work be completed before the start of breeding season on March 23rd, and it delayed prescribed burning until after the end of breeding season (September 15th). As a result, we had to change our projected spring burn into a fall burn, when fewer crews are available and air quality restrictions increase, so that the actual prescribed burn differed from the one originally planned. Treatment timeframes are also limited by *P. ramorum*'s rapid spread. Effective control requires an excellent early detection and rapid response team. Even with good early detection, however, quick management response can be limited by lengthy contracting and environmental review processes that do not support emergency action. At this point in California, the presence of *P. ramorum* in forested areas has not constituted an emergency situation.

Besides dealing with the difficulties of timing treatments, land managers performing operations designed to manage *P. ramorum* must also consider sanitation to avoid pathogen transmission off the site. Cushman and others (this volume) and Tjosvold and others (2002) demonstrated that soil taken from hikers' shoes contained *P. ramorum* inoculum, while Cooper and Cushman (2006) found that *P. ramorum* incidence is higher in areas with high human visitation and activity. For the silvicultural treatments detailed in this study, we mandated that crews scrub the bottoms of their boots and wash them in a 10 percent bleach solution before returning to their home bases as well as thoroughly cleaning chainsaws, brush chippers, chaps, and other equipment with compressed air, brushes, brooms, and manual inspection for clinging foliage. Additionally, landowners of some of the parcels on which treatment occurred improved the parking areas for crew vehicles by lining the parking areas with new gravel or rock so that the vehicles could avoid parking in mud and crews would have a cleaner surface for their personal sanitation. Land managers planning *P. ramorum* management operations should take into account the additional time and expense involved in these kinds of activities.

Crews

Phytophthora ramorum management, in our experience, is only effective to the degree that crews are brought up to speed and supervised effectively. For all crews, there is a learning curve associated with each project because the treatments utilized non-traditional prescriptions and because prescriptions varied between sites. We were not able to use the same crew on each of the project areas, for scheduling, financial and/ or political reasons. As a result, costs varied between projects (table 1). For example, the California Department of Corrections has low-cost fire crews that are available for work on public properties, but these crews are not available for use on private properties, nor are they are available in all locations in California. While these crews were less expensive than private crews, they did require more on-site supervision by project designers, the costs of which are not reflected in table 1.

Costs

As with all silvicultural treatments, costs vary with the complexity of the project. In our situation, costs probably increased by the addition of sanitation requirements and the development of non-traditional prescriptions; however, increasing experience with treatment implementation on the part of supervisors and crews will probably lessen these costs over time. The most obvious cost at this time is that associated with processing large infected bay trees and the unknown status of new bay and tanoak sprouts. We sought to reduce these costs by testing two alternatives to cutting trees down by chainsaw. In neither situation did we find a satisfactory alternative. In the case of the herbicide work, bay trees within all size classes are still alive fourteen months after treatment, albeit with yellow leaves and thinned crowns. We are still able to recover the pathogen from these leaves, and presumably the spores can still spread the pathogen. More information is needed about whether there are socially acceptable and biologically effective alternative herbicide(s) to kill infected bay trees. Additionally, it has been 10 months since large bay trees were girdled, and their crowns show no difference in color and vigor from crowns of untreated trees. Use of herbicides or girdling by chainsaw to kill bay trees may be appropriate in areas where the pathogen is not present or where a rapid (i.e. <4 month) treatment effect is not needed.

The most complex treatment in this study, the treatment involving removal of all bay and tanoak trees, is probably too expensive for most land managers to implement in the bulk of the *P. ramorum* infested area of California. However, where expectations for control are higher, especially where the infested areas are small (<2 ha), the cost may be bearable. It is important to note that these treatments produced no commercial products. While we did leave logs on site that could be utilized for firewood, there is potential risk for pathogen transmission in the movement of infected tanoak logs (Parke and others 2007), so firewood use should not be considered.

Public Perceptions

Finally, the land manager dealing with *P. ramorum* must consider public perceptions of treatment. Our active management of *P. ramorum* reinforces our educational message concerning the pathogen in the north coast of California by demonstrating that landowners and land managers are concerned enough about the spread of the disease to take action. This area of California features a relatively high level of

ecological knowledge and concern among its residents; therefore, most of the landowners that we approached with suggestions for treatment offered enthusiastic support, which was augmented by an active stakeholder consultation process, quick notification to landowners of disease detections on their properties, and regular communication with neighbors. Additionally, actively managing this pathogen often foregrounds more general forest health issues for landowners and motivates them to practice better stewardship of their properties. These silvicultural management activities facilitated many non-disease-related goals, such as road improvement, conifer restoration, and fuels reduction on treated properties.

Although we have discovered that landowners display great enthusiasm for *P. ramorum* management, they do not equally accept all possibilities for treatment. What is acceptable for a landowner usually depends on the particular goals and objectives for ecosystem management on that property, some of these codified by law or agency policy. Forests in this area under California Department of State Parks ownership, for example, could not be treated with herbicides; likewise, we have encountered some private owners averse to herbicide use on their properties, partly because of community antipathy toward such use. Conversely, some other owners accept herbicide killing of hardwoods as an accelerated route toward conifer restoration on their land. Treatment on some properties may preclude prescribed burning because of location and air quality concerns, while other landowners may impose maximum diameter limits on the removal of bay and tanoak trees. Since public opinion of recommended treatments is a determining condition of future management work, this must be taken into account when judging treatment effectiveness.

Conclusions

In summary, evaluating treatment effectiveness will take several years and must consider a variety of biological, social, and economic factors, some of which are measurable now, while others are not. Of the treatments tested, costs were generally quite variable (e.g. 620 to $8,645/ ha). Treatment implementation was constrained by a variety of wildlife issues and landowner preferences. On the least expensive side, the herbicide and girdling treatments tested to control bay trees were not effective in the desired timeframe; as a result, testing of additional bay control strategies is needed. The effectiveness of the most expensive treatment, full removal of infected bay and tanoak trees with broadcast burning, is not known to date, but this treatment does show the greatest quality and uniformity of host removal. While the costs of this treatment are very high, it may be palatable in areas of exceedingly high risk or where standards for success are high. At this point it is generally too early to recommend one treatment type versus another. However, it is clear that if the pathogen is left unmanaged, the area infested and impacted in California will continue to grow (Rizzo 2007).

Acknowledgments

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Contingency Planning for *Phytophthora ramorum* Outbreaks: Progress Report Work Package 7, EU RAPRA Project[1]

M.H.C.G. Steeghs[2]

Abstract

As part of the EU project "Risk analysis for *Phytophthora ramorum,* a recently recognised pathogen threat to Europe and the cause of Sudden Oak Death in the USA" (acronym RAPRA) outbreak scenarios are defined and existing strategies for eradication and containment of *Phytophthora ramorum* evaluated. Based on the current knowledge about disease epidemiology three types of outbreaks have been identified: the rhododendron system in northern Europe, a hypothetical system in southern Europe, and the nursery system. The logistic curve is suggested as a model to describe the geographical spread of *P. ramorum*.

Evaluation of existing strategies is subdivided into three categories: measures to prevent introduction, measures at nurseries and measures in the natural and semi-natural environment. Up until now there have been no indications that introductions have occurred in the EU from the U.S. However, in the U.S. there have been findings of the A1 mating type-European lineage at nurseries in Oregon and Washington State, suggesting another introduction has occurred at some time, either from Europe or another origin. Generally the measures at the nurseries are adequate, provided they are fully implemented. Since they have been in place there has been a sharp decline in the incidence of positive findings in the U.K. and the Netherlands. There are shortcomings in the measures for growing media, contact with the environment and uncertainties regarding the effect of contaminated irrigation water. Also information on the period before symptom development and optimal timing of inspection is incomplete. Evaluation of the measures in natural and semi-natural environment in the EU shows that if only a few plants are infected, eradication can be achieved. However, if large areas of rhododendron are infected control measures are required over a number of years and often containment is the maximum control attainable. Until now in the EU all infestations are in or linked to rhododendron. *P. ramorum* can be managed in the EU by managing rhododendron.

Key words: *Phytophthora ramorum*, outbreak scenario, evaluation, eradication, containment.

[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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Introduction

The overall aim of the EU project "Risk analysis for *Phytophthora ramorum*, a recently recognised pathogen threat to Europe and the cause of Sudden Oak Death in the USA" (acronym RAPRA) is to develop a European pest risk analysis for American (U.S.) and European (EU) isolates of *P. ramorum*. The results should allow a review of the current EU phytosanitary policy. The tasks of the RAPRA project are clustered in work packages. In Work Package 7, "contingency planning for *Phytophthora ramorum* outbreaks", outbreak scenarios are defined and existing strategies for eradication and containment evaluated. With the production of technical guidelines for eradication and containment the work package will be completed. Work Package 7 is jointly carried out by Forest Research U.K., Central Science Laboratories U.K., Plant Health-Department for Environment Food and Rural Affairs, U.K., Federal Biological Research Centre for Agriculture and Forestry Germany, United States Department of Agriculture-Forest Service (USDA-FS) and the Netherlands Plant Protection Service.

Outbreak Scenarios

The outbreak scenarios are designed for European circumstances to assess the potential economic and environmental impacts by *P. ramorum* **(**Kehlenbeck 2007). Information on the actual situation was obtained through a questionnaire to the EU member states, and also from the annual *P. ramorum* survey reports submitted by the member states to the EU. Additional data was also provided by the U.K. and the Netherlands. Based on this information and knowledge of the principles involved in outbreaks (for example, host type, conditions for establishment and means of dispersal), three outbreak scenarios have been defined.

- The rhododendron system; exemplifies the situation in the temperate zone of northwest Europe with rhododendron as sole foliar (sporulating) host and a number of tree end hosts. Dispersal will take place within and between patches with sporulating host plants.
- A hypothetical system that exemplifies the situation in the Mediterranean climate zone of southwest Europe, with foliar (sporulating) tree and non-tree hosts and a number of tree and non-tree end hosts. The system is based on the assumption that there will be a driving force in the form of a sporulating foliar tree host. The spread can be over relatively large distances.
- A nursery system that exemplifies a generalised nursery with a variety of foliar and non-foliar host species arranged in uniformly planted beds in protected or semi-protected environment. The plants grow under favourable conditions. The disease spread can be over short to very large distances.

The nursery system and the two natural systems considered are interrelated via trade linked introduction pathways.

The logistic curve (S-curve) having an approximately exponential increase at the initial stage and a slow down and eventually a halt at later stage can be used as a model to describe the geographical spread of *P. ramorum* throughout an area with

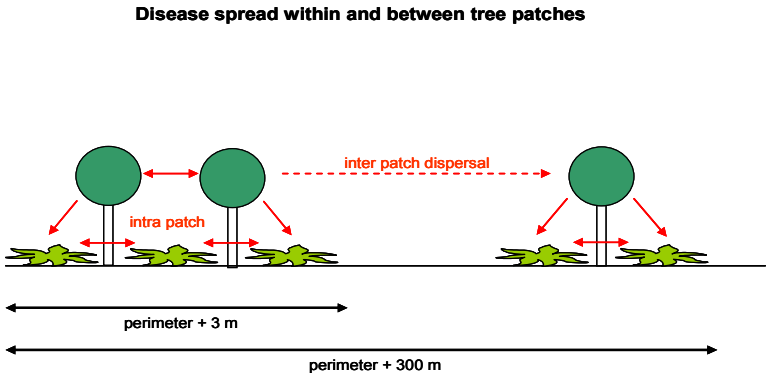

Figure 1—Schematic presentation of the hypothetical system in semi-open landscapes and forest with patches of foliar tree (e.g. *Quercus ilex*) and understory species and tree and non-tree end hosts; intrapatch disease spread by regular short distance dispersal of sporangia (eg. rainsplash); interpatch disease spread by incidental medium distance dispersal of inoculum (e.g.storm/rain splash).

patches of rhododendron or other sporulating foliar hosts capable of driving an epidemic under favourable climatic conditions. It is assumed that from the nurseries, infested plants are introduced in patches with rhododendron or other foliar hosts. The percentage of infested patches depends on the distance between the patches but also on the effectiveness of the measures (plant material free). Without measures within a patch, ultimately all the sporulating foliar hosts in that patch capable of maintaining an epidemic will be infested. The infestation level of other host plants will depend on the environmental circumstances. Until now the incidence of findings in other host plants is low.

Evaluation of Existing Strategies

The evaluation is subdivided into measures to prevent introduction, measures at nurseries and measures in the natural and semi-natural environment.

Preventing Introduction

In general the measures are directed to prevent introduction associated with the importation of susceptible plants, wood and bark. The danger of recombination between U.S. and EU isolates of *P. ramorum* increases the fear of introductions between North America and the EU. Up until now there have been no indications that introductions have occurred in the EU from the U.S. However, in the U.S. there have been findings of the A1 mating type-European lineage at nurseries in Oregon and Washington State and more recently in California (COMTF 2007), suggesting introductions have occurred at some time, either from Europe or another origin. Besides the U.S. and the EU 50 countries (IPPC 2007) have *P. ramorum* on their regulated pests lists or mentioned in their legislation.

Nurseries

The necessary information for the evaluation of the measures at the nurseries in the EU was obtained through questionnaires to the Member States, through in-depth studies at nurseries, from the EU *Phytophthora ramorum* working Group and in a

limited way from research results. Evaluation of the measures in the U.S. was confined to using the information to support the evaluation of the EU measures. Information on U.S. measures was obtained from the Animal and Plant Health Inspection Service (APHIS).

The results of the evaluation in Work Package 7 are very much in line with the results of the EU *Phytophthora ramorum* Working Group that met in May 2006. The Working Group convened to evaluate briefly the Member State survey and RAPRA research results and to make recommendations for improvement of the EU Commission Decision[3].

The measures are generally adequate when implemented fully. This is demonstrated by the sharp decline in the number of positive findings at the nurseries in two Member States. In England and Wales, from 2003 to 2006, *P. ramorum* positive inspections declined from 3 percent to less than 1 percent. In the Netherlands, over a similar time scale, findings reduced from 4 percent of the nurseries infected to less than 0.5 percent. However the number of infestations found in marketed lots by the receiving countries is not always in concurrence with the decline found at the producer's nurseries. One of the reasons might be the marketing of asymptomatic but infected plants.

There are shortcomings:

- Information from different Member States in the EU but also from the U.S. indicates infested soil and debris is a cause of infestations.
- Infection of nursery plants through contact with infestations in the semi-natural environment, but also inadequate inspections leading to the introduction of the pathogen at the nursery via infected planting material are reported as important factors.
- Infection spread via irrigation remains a point of attention. Research (Werres and others 2007) clearly indicates that *P. ramorum* can be spread with contaminated water and that infection via overhead irrigation is possible. Further studies are needed to investigate the conditions favouring infection and symptom development.

These points have also been raised at the EU Working Group, resulting in recommendations for adaptations to the EU regulation and technical guidelines (Slawson and others 2006). The EU Standing Committee on Plant Health accepted the recommendations of the EU Working Group in February 2007.

The evaluation of the measures currently in place made it clear that more research is required on the likelihood and circumstances under which infection can be initiated by infested soil and irrigation water. Also more information is needed on the lag period before symptom development and on the optimum timing for inspection.

[3] COMMISSION DECISION of 19 September 2002 to prevent the introduction into and spread within the Community of *Phytophthora ramorum* Werres, De Cock & Man in't Veld *sp. nov.* (notified under document number C – 2002 3380).

Natural and Semi-natural Environment

Until now in the EU all infestations are in or linked to rhododendrons. The EU Commission Decision requires only that the harmful organism should be contained. The EU Working Group on *Phytophthora ramorum* worked out the approach in technical guidelines (Slawson and others 2006**)**.

Experiences in both the U.K. and the Netherlands have shown that if only a few plants are infected, eradication can be achieved successfully. However where large areas of rhododendrons are infected, experience has shown that control measures are required over a number of years and even then, it is uncertain whether or not complete eradication is achievable (Slawson 2006). The inputs required for eradication are often substantial and of such long duration that they cannot be met.

Less effective but cheaper containment measures such as pruning back infected plants, chipping the debris and leaving this on site are often financially and socially more acceptable. Due to its acceptability, containment measures can often contribute more to the prevention of the spread than eradication measures.

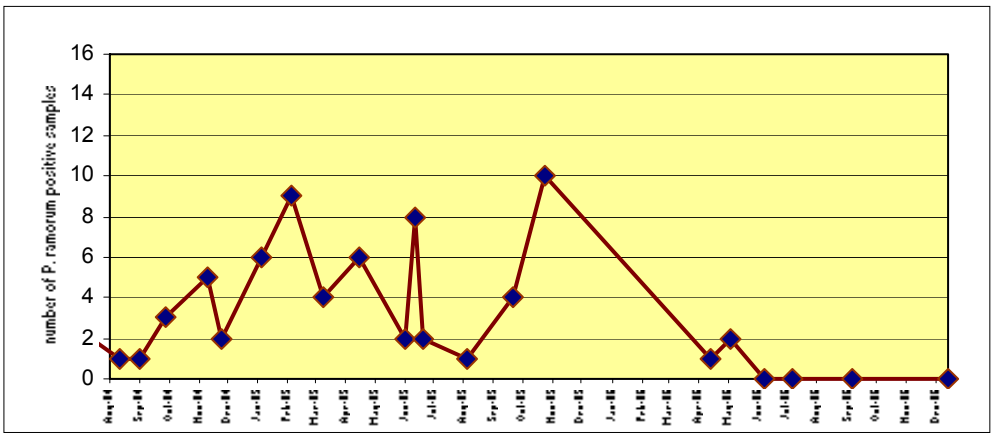

Figure 2— Survival of *Phytophthtora ramorum* in woodchips.

In an experiment in the Netherlands to monitor the persistence of *P. ramorum* after infected rhododendrons were cut down and chipped, it took 2 years before the pathogen could no longer be detected in the previously infested woodchips. An earlier trial in 2003/2004 in the Netherlands proved that it took 1.5 years before the pathogen could not be detected in the soil after removing infected rhododendrons. The additional risk of leaving infested woodchips at the site is limited, considering the short extra period the pathogen could be detected.

It is accepted in both the Netherlands and the U.K. that in cases where eradication cannot be achieved containment measures are taken, with the overall aim of preventing the spread of *P. ramorum*. In both countries it is concluded that these containment measures reduce considerably the risk of the pathogen spreading. Such containment measures in the natural and semi-natural environments are often very similar to the normal maintenance of intentionally planted rhododendrons.

The experiences in southern Oregon also show that eradication is difficult in the natural environment. Despite an intensive eradication campaign, over the last 2 years the number of newly found infested trees has increased (Kanaskie this volume).

However, in the infested area in northern California (Humboldt County) the spread has been considerable compared to the Oregon infested area. It is assumed that without treatment spread in Oregon would be similar to northern California. Maybe the conclusion is that even containment may not be possible and that slow in spread rate is the best that is achievable under conditions of established widespread outbreaks. In Humboldt County trials have been implemented to find an acceptable and affordable way to manage the disease. The trials include burning and removal of bay laurel and tanoak (COMTF 2006), but only after two years will it be possible to say whether the approach is successful. Undoubtedly, the impact of *P. ramorum* on the environment in California U.S. is much more serious than in the EU due to its conducive environment as well as a continuum of highly susceptible bole (tree) host and tree sporulators.

General Conclusions

The measures to prevent introduction of the pathogen in the EU have been successful. However the findings of A1 mating type-European lineage in the U.S. suggest an introduction at some time from Europe or another origin.

The measures at the nurseries in Europe are in general adequate. There has been a sharp decline in positive findings in the U.K. and the Netherlands. Shortcomings are reported in the measures for growing media and contact with the environment. Further studies are needed on the effect of irrigation water and there is lack of information on the period between infection and symptom development and on the optimal timing of inspection.

Until now in the EU all infestations found in the natural environment are in or linked to rhododendrons. Based on the information from the U.S. and the EU, for Europe some general conclusions can be drawn about strategies for eradication and containment. With small outbreaks, consisting of only a few plants, eradication can be successful as has been demonstrated in nurseries and the natural environment. If a larger outbreak has established itself however, often containment is the maximum achievable. Nevertheless, for Europe, *Phytophthora ramorum* can be managed by managing rhododendron. Considering the difficulties with eradication and containment, our main aim has to remain preventing introductions in new areas.

Acknowledgements

I would like to use the opportunity to express my gratitude and appreciation for the contributions of all the co-workers in the Work Package 7 of the EU RAPRA project named below. In the above presentation I have made use extensively of their specific contributions.

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Posters

Six Years of Aerial and Ground Monitoring Surveys for Sudden Oak Death in California[1]

Lisa Bell,[2] Jeff Mai,[2] Zachary Heath,[2] Erik Haunreiter,[3] and Lisa M. Fischer[2]

Abstract

Aerial surveys have been conducted since 2001 to map recent hardwood mortality and consequently target ground visits for detection of *Phytophthora ramorum*, the pathogen that causes sudden oak death (SOD). Each year the aerial and ground surveys monitored much of California's forests at risk for SOD resulting in new maps of hardwood mortality, detection of new *P. ramorum* infestations, and identification of areas unlikely to be infested by *P. ramorum*. Nearly 50,000 miles of California forest and woodland were flown by helicopter and fixed-wing aircraft over six years of aerial surveys. Of the 2,687 mortality polygons mapped, 669 were visited by field crews to check for SOD symptoms. Thirty-eight new confirmations of *P. ramorum* resulted from these surveys. The 2006 surveys covered 11 counties and resulted in 10 new detections. SOD is currently found in 14 counties in California, mostly on private land. Using risk maps combined with aerial survey data, areas where SOD is most likely to become established can be identified for monitoring and management. For more information on the monitoring surveys go to http://www.fs.fed.us/r5/spf/fhp/fhm.

Key words: Sudden oak death, *Phytophthora ramorum*, aerial survey, monitoring.

[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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Effectiveness of Fungicides in Protecting Conifers and Rhododendrons From *Phytophthora ramorum*[1]

Gary A. Chastagner,[2] Annie DeBauw,[2] Kathy Riley,[2] and Norm Dart[2]

Abstract

The effectiveness of 19 fungicides in protecting noble fir, grand fir, and *Rhododendron* x 'Nova Zembla' foliage from *P. ramorum* was tested. The tops of conifer seedlings with newly emerging shoots and mature rhododendron leaves were collected from treated plants 7 days after drench applications or 1 day after foliar applications. Detached seedling tops and leaves were then placed on moistened filter paper in Petri dishes and inoculated. Zoospores from three European genotype isolates of the A1 mating type (EU1 lineage) and three NA1 lineage isolates of the A2 mating type were mixed to create two inoculum sources representing the A1 and A2 mating types for the conifer tests. Detached rhododendron leaves were inoculated with suspensions of zoospores from a EU1 A1 mating type rhododendron isolate and a North American genotype NA1 A2 mating type rhododendron isolate. The rhododendron leaves were inoculated by pipetting three 10 ul drops of zoospore suspension onto the lower leaf surface on each side of the leaf midrib. The leaf tissue was injured beneath 3 drops on one side of the leaf midrib using an insect pin. The tissues beneath the drops on the other side of the leaf were left unwounded. Checks included inoculated and non-inoculated conifer tops and rhododendron leaves that had been treated with plain water. Following inoculation, the Petri dishes were incubated for 7 days at 19°C in plastic tubs. Fungicide efficacy on the conifers was quantified by calculating the percent of visibly diseased shoots on each seedling top. Fungicide efficacy on the rhododendron leaves was quantified by counting the number of inoculation sites that developed symptoms and the area of each resulting lesion using ASSESS. No disease developed on any of the non-inoculated conifer or rhododendron checks. On the conifers, ANOVA analysis indicated that genotype and treatment ($P<0.0001$) were the only variables that had a significant affect on infection. Overall, the seedling tops inoculated with the A1 mating type had significantly fewer infected shoots than the seedlings inoculated with the A2 mating type. Applications of Dithane, Gavel, Stature, Ranman, Maneb, Polyram, Fenstar, Daconil Ultrex, V-10161, and Insignia were the most effective fungicides in reducing infection of the conifer shoots by both the A1 and A2 mating types of *P. ramorum*. On rhododendron, ANOVA analysis indicated that wounding and treatment ($P = 0.001$) were the only variables that had significant effects on the number of infected inoculation sites and lesion size. Overall, fewer fungicides were effective in reducing disease development on the wounded leaf surfaces. Maneb, Gavel, Subdue MAXX, Ranman (3 oz), Stature DM and Dithane were the most effective treatments in controlling disease development on both the wounded and non-wounded rhododendron leaves.

Key words: *Phytophthora ramorum*, disease management, fungicides, control, conifers, rhododendrons.

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Introduction

The exotic plant pathogen, *Phytophthora ramorum,* has the potential to infect a number of commonly grown nursery crops in the Pacific Northwest, which is a major production area for ornamental and conifer nursery stock. Nurseries currently utilize a number of cultural practices, such as isolation of production blocks, drainage, treatment of recirculated water, and irrigation management, to prevent various *Phytophthora* diseases. There are a number of systemic and contact fungicides that are also often used to help control *Phytophthora* diseases on nursery crops.

Limited information is available regarding the effectiveness of many of these fungicides in protecting plants from *P. ramorum*. During 2005, we conducted two trials to evaluate the effectiveness of 19 fungicides in protecting noble fir (*Abies procera*), grand fir (*A. grandis*), and *Rhododendron* x 'Nova Zembla' foliage from infection by *P. ramorum* (table 1).

Table 1—List of fungicides tested

Trade name	Common name	Rate of product/100 gal	Application method[1]
Aliette	Fosetyl AL	5 lbs	F
Alude	Phos. acid gen.	1 qt	D
Biophos	Phos. acid gen.	2%	D
Champ Formula 2F	Copper hydroxide	1.33 pts	F
Daconil Ultrex	Chlorothalonil	1.4 lbs	F
Dithane 75 DF	Mancozeb	2 lbs	F
Fenstar	Fenamidone	14 & 28 oz	F
Gavel 75 DF	Mancozeb + zoxamide	2 lbs	F
Insignia 20W	Pyraclostrobin	16 & 40 oz	F
Magellan	Phos. acid gen.	80 fl oz	D
Maneb 75 DF	Maneb	2 lbs	F
Polyram 80 DF	Metiram	2 lbs	F
Ranman	Cyazofamid	3 & 6 oz	F
Rhapsody	Bacillus subtilis	1, 1.5, & 2%	F
Stature DM	Dimethomorph	6.4 & 12.8 oz	F
Subdue MAXX	Mefenoxam	2 fl oz	D
TM-473	Fluoxastrobin	5 oz	F
V-10161	Potassium phosphite	1.4 oz ai	F
Vital	Phos. acid gen.	4 pt	D

[1]Application method: F = foliar and D = drench.

Materials and Methods

Conifer Test

Frozen field-grown noble fir (size 1-0) and container-grown grand fir seedlings (Styro 2A plugs) were transplanted into Ray Leach "Conetainers"™ and stored in a cooler at 3°C. In order to force bud break, seedlings were placed in a warm greenhouse until the new shoots at the top of the seedling had reached 1 to 2 cm in length. Drench treatments were applied 7 days prior to inoculation. The foliar fungicide treatments were applied with a backpack sprayer to seedling tops 6 days after the drench applications. The following day, tops of both the drenched and foliar-treated seedlings were cut off and placed on moistened filter paper (Whatman #2) in a Petri dish. The detached noble fir and grand fir seedling tops had an average of 6.7 and 6.9 emerging shoots per top, respectively.

Two zoospore suspensions, representing the European (EU1 lineage A1 mating type) and North American (NA1 lineage A2 mating type) genotypes of *P. ramorum* were prepared and used to inoculate the seedling material in each petri plate. Three isolates of each genotype were mixed to provide inoculum. The EU1 A1 isolates used were 03-74-1 (from Oregon, host *Pieris japonica*), 03-74-2 (Oregon, host *Viburnum x bodnantense* 'Dawn') and 03-74-N11-A (Oregon, host *Rhododendron* x 'Unique'). The NA1 A2 isolates were originally from Oregon tanoak (No. 2018.1, 2027.1, and 2109). Spore counts of each suspension were calculated using a hemacytometer. The EU1 A1 spore count was 378,000 spores/ml and the A2 was 350,500 spores/ml. The spore suspensions were applied using an airbrush sprayer. The non-inoculated check tops were sprayed with water only. Following inoculation, the Petri plates were placed in sealed tubs and incubated for 7 days at 19°C.

Treatment effectiveness was determined by counting the number of diseased emerging shoots out of a maximum of eight buds on each seedling top. Isolations were done from representative symptomatic shoots in each plate onto CARP medium and then subcultured to 1/3 V8 medium for identification to ensure symptoms were associated with infection by *P. ramorum*.

This experiment was a randomized split block design with two hosts (noble and grand fir), two *P. ramorum* genotypes (A1 and A2), and 27 treatments. Treatments included inoculated and non-inoculated checks. There were three noble fir and four grand fir seedlings per treatment and genotype. Data were analyzed using a one-way ANOVA (GLM SAS). Treatment means were separated using Tukey's Studentized Range (HSD) Test.

Rhododendron *Test*

Rhododendron x 'Nova Zembla' plants grown outdoors in 3.8 l (1 gal) pots were used during this test. One day after the foliar applications and 7 days after the drench applications, two leaves were removed from each plant. These leaves were trimmed and each leaf was placed with its lower surface up on moistened filter paper in a Petri dish. A total of five plants were used per treatment.

Suspensions of zoospores from a single isolate of each genotype were prepared. The EU1 A1 isolate was 03-74-N11-A (from Oregon, host *Rhododendron* x 'Unique') and the NA1 A2 isolate was 03-74-N10-A (Oregon, *Rhododendron* x 'Unique'). Spore counts were determined using a hemacytometer and were 688,000 spores/ml

and 668,000 spores/ml for EU1 A1 and NA1 A2, respectively. Three 10 ul drops of inoculum were placed on each side of the leaf midrib. An insect pin was then used to injure the leaf tissue beneath the 3 drops on one side of the leaf. The tissue beneath the drops on the other side of the leaf was not wounded. Following inoculation, the Petri plates were placed in sealed tubs and incubated for 7 days at 19°C.

Treatment effectiveness was determined by counting the number of inoculation sites that developed symptoms and measuring the area of each resulting lesion using the APS ASSESS Image Analysis software. Isolations were done from representative symptomatic lesions onto CARP medium and subcultured to 1/3 V8 medium to ensure that *P. ramorum* was the cause of infection.

This experiment was a split-split plot design with plants nested in treatments and split by mating type (EU1 A1 and NA1 A2) and wounding (wounded and non-wounded). Treatments included inoculated and non-inoculated checks. Data were analyzed using ANOVA (GLM SAS). Treatment means were separated using Tukey's Studentized Range (HSD) Test.

Results and Discussion

Conifer Test

Genotype ($P < 0.0001$) and treatment ($P < 0.0001$) were the only variables that were significant in the ANOVA. Overall, the seedlings tops inoculated with the EU1 A1 genotype had significantly fewer diseased shoots than the seedlings inoculated with the NA1 A2 genotype (data not shown). No symptoms developed on any of the non-inoculated seedling tops.

For seedlings inoculated with the EU1 A1 genotype, the numbers of diseased shoots on several of the treated seedlings were not significantly different than the non-inoculated checks (table 2). These treatments included Dithane, Gavel, Stature, Ranman, Maneb, Polyram, Fenstar, Daconil Ultrex, V-10161, Insignia, and Subdue MAXX. None of the other fungicides reduced the number of diseased shoots compared to the inoculated checks.

When inoculated with the NA1 A2 genotype, the number of diseased shoots on seedlings treated with Dithane, Gavel, Stature, Ranman, Maneb, Polyram, Fenstar, Daconil Ultrex, V-10161, and Insignia (1.18 l = 40 oz) also did not differ significantly from the non-inoculated checks (table 2). None of the other fungicides reduced infections compared to the inoculated checks.

Rhododendron *Test*

ANOVA analysis indicated that wounding and treatments were the only variables that had significant effects on the number of inoculation sites that developed symptoms and lesion size. Since genotype had no effect on symptom development and lesion size, separate analyses were done on the wounded and non-wounded inoculation sites to assess treatment difference using the combined EU1 A1 and NA1 A2 data.

Table 2—Effect of fungicide treatments on the average number of infected shoots on the detached noble and grand fir seedling tops

Treatment	Rate/100 gal	Application method[1]	*P. ramorum* genotype A1	*P. ramorum* genotype A2
Vital	4 pt	D	3.3 abc[2]	5.7 a
Rhapsody	2%	F	2.9 bcde	5.6 a
Magellan	80 fl oz	D	3.0 abcd	5.6 a
Inoc. Check	n/a	n/a	4.8 a	5.6 a
Alude	1 qt	D	2.6 cdef	5.3 a
Biophos	2%	D	4.4 ab	5.1 ab
Rhapsody	1%	F	4.7 a	5.0 abc
Rhapsody	1.5%	F	4.6 ab	4.4 abcd
Insignia 20W	16 oz	F	1.3 defgh	3.3 bcde
Champ Formula 2F	1.33 pts	F	2.1 cdefg	3.0 cdef
Aliette	5 lbs	F	3.0 abcd	2.9 def
TM-473	5 oz	F	3.3 abc	2.7 defg
Subdue MAXX	2 fl oz	D	0.1 h	2.6 defgh
Insignia 20W	40 oz	F	1.4 defgh	1.7 efghi
V-10161	1.4 oz ai	F	1.0 fgh	1.6 efghi
Fenstar	14 oz	F	0.3 h	1.4 efghi
Daconil Ultrex	1.4 lbs	F	1.1 efgh	1.4 efghi
Fenstar	28 oz	F	0.0 h	1.1 efghi
Ranman	3 oz	F	0.3 h	1.1 efghi
Polyram 80 DF	2 lbs	F	0.3 h	1.0 fghi
Stature DM	6.4 oz	F	1.1 efgh	1.0 fghi
Maneb 75 DF	2 lbs	F	0.0 h	0.6 ghi
Ranman	6 oz	F	0.0 h	0.4 hi
Stature DM	12.8 oz	F	0.0 h	0.4 hi
Gavel 75 DF	2 lbs	F	0.6 gh	0.1 i
Dithane 75 DF	2 lbs	F	0.0 h	0.0 i
Non-inoc. Check	n/a	n/a	0.0 h	0.0 i

[1]Drench (D) or foliar (F) application.
[2]Numbers in columns followed by the same letter are not significantly different, P=0.05, Duncan's Multiple Range Test.

On the non-wounded inoculation sites, numbers of symptomatic inoculation sites and lesion area on leaves treated with Maneb, Fenstar, Gavel, Dithane, Polyram, Subdue MAXX, Ranman, Daconil Ultrex, Stature (363.7 ml = 12.8 oz), and Insignia (1.18 l = 40 oz) were not significantly different than the number of symptomatic inoculation sites and lesion area on the non-inoculated checks, which were zeros (table 3). Although the lesion areas on the leaves treated with Insignia (473.2 ml = 16 oz), Stature (189.3 ml = 6.4 oz) and V-10161 were also not different that those on the non-inoculated checks, the number of symptomatic inoculation sites was higher and did not differ from the inoculated checks. TM-473 provided intermediate reduction in lesion area, but had no effect on the number of inoculation sites that developed symptoms. The other fungicides (Magellan, Rhapsody, Aliette, Vital, Biophos, Alude, and Champ) neither significantly reduced the number of inoculation sites that developed symptoms nor did they reduce lesion area compared to the inoculated checks.

Table 3—Effectiveness of fungicides in limiting infection and lesion size on non-wounded leaves inoculated with *Phytophthora ramorum*

Treatment	Rate	Application Method[1]	No. infected sites[2]	Lesion area[3]
Magellan	80 fl oz	D	3.0 a[4]	127.5 a
Rhapsody	1.5% formula	F	2.8 ab	118.0 abc
Rhapsody	1% formula	F	2.8 ab	98.8 abcd
Inoc. Check	n/a	n/a	2.7 abc	123.0 ab
Aliette	5 lbs	F	2.7 abc	87.2 cd
Vital	4 pt	D	2.5 abc	79.9 de
Biophos	2% formula	D	2.5 abc	67.5 defg
Alude	1 qt	D	2.4 abc	94.9 abcd
TM-473	5 oz	F	2.1 bcd	48.1 efgh
Champ Formula 2F	1.33 pts	F	1.9 cde	72.3 def
Rhapsody	2% formula	F	1.5 def	89.1 bcd
Insignia 20W	16 oz	F	1.4 defg	33.7 ghi
Stature DM	6.4 oz	F	1.3 defgh	43.0 fgh
V-10161	1.4 oz ai	F	1.3 defgh	27.5 hi
Insignia 20W	40 oz	F	1.1 efghi	20.6 hi
Stature DM	12.8 oz	F	0.9 fghij	27.2 hi
Daconil Ultrex	1.4 lbs	F	0.9 fghij	20.6 hi
Ranman	3 oz	F	0.9 fghij	18.9 hi
Ranman	6 oz	F	0.7 fghij	18.0 hi
Fenstar	28 oz	F	0.6 ghij	17.7 hi
Subdue MAXX	2 fl oz	D	0.6 ghij	3.0 i
Polyram 80 DF	2 lbs	F	0.5 hij	11.9 hi
Dithane 75 DF	2 lbs	F	0.4 ij	14.9 hi
Gavel 75 DF	2 lbs	F	0.1 j	0.0 i
Fenstar	14 oz	F	0.0 j	0.0 i
Maneb 75 DF	2 lbs	F	0.0 j	0.0 i
Non-inoc. Check	n/a	n/a	0.0 j	0.0 i

[1]D = Drench and F = Foliar spray.

[2]Average number out of 3 inoculated sites per leaf.

[3]Average lesion area (mm^2) per 3 inoculation sites per leaf.

[4]Numbers followed by the same letter are not significantly different, P=0.05, Duncan's Multiple Range Test.

Fewer fungicides reduced disease development on the wounded leaves (table 4). The numbers of inoculation sites that developed symptoms and lesion area on leaves treated with Maneb, Gavel, Subdue MAXX, Ranman (88.7 ml = 3 oz), Stature DM and Dithane were not significantly different than the number of symptomatic inoculation sites and lesion area on the non-inoculated checks, which were zeros. Although the lesion area on the leaves treated with Polyram, V-10161, Fenstar, Ranman (177.4 ml = 6 oz), Insignia, Daconil Ultrex, and TM-473 was not significantly different than the non-inoculated checks, these fungicides did not significantly reduce the number of inoculation sites that developed symptoms. Applications of Rhapsody, Magellan, Alude, Biophos, Aliette, Vital, and Champ neither significantly reduce the number of inoculation sites that developed symptoms nor did they reduce lesion area compared to the inoculated checks.

Table 4—Effectiveness of fungicides in limiting infection and lesion size on wounded leaves inoculated with *Phytophthora ramorum*

Treatment	Rate	Application Method[1]	No. infected sites[2]	Lesion area[3]
Rhapsody	**1.5% formula**	F	3.0 a[4]	141.9 a
Rhapsody	1% formula	F	3.0 a	102.5 abcd
Inoc. Check	n/a	n/a	2.9 ab	131.3 ab
Magellan	80 fl oz	D	2.8 ab	119.2 ab
Alude	1 qt	D	2.8 ab	105.8 abc
Biophos	2% formula	D	2.7 abc	75.9 cdef
TM-473	5 oz	F	2.7 abc	50.0 efgh
Rhapsody	2% formula	F	2.6 abc	103.8 abcd
Aliette	5 lbs	F	2.6 abc	70.2 cdefg
Insignia 20W	16 oz	F	2.5 abc	52.1 efg
Fenstar	14 oz	F	2.4 abc	26.9 ghij
Daconil Ultrex	1.4 lbs	F	2.3 abcd	52.5 efg
Vital	4 pt	D	2.2 abcde	62.0 defg
Insignia 20W	40 oz	F	2.2 abcde	41.7 fghij
Champ Formula 2F	1.33 pts	F	2.1 abcde	91.4 bcde
Ranman	6 oz	F	1.9 bcdef	35.4 fghij
Fenstar	28 oz	F	1.7 cdefg	42.3 fghij
V-10161	1.4 oz ai	F	1.7 cdefg	34.4 fghij
Polyram 80 DF	2 lbs	F	1.7 cdefg	34.0 fghij
Dithane 75 DF	2 lbs	F	1.4 defgh	56.5 efg
Stature DM	6.4 oz	F	1.3 efgh	45.7 fghi
Stature DM	12.8 oz	F	1.1 fghi	38.7 fghij
Ranman	3 oz	F	1.1 fghi	27.1 ghij
Subdue MAXX	2 fl oz	D	0.8 ghij	3.2 ij
Gavel 75 DF	2 lbs	F	0.5 hij	6.0 hij
Maneb 75 DF	2 lbs	F	0.3 ij	4.3 ij
Non-inoc. Check	n/a	n/a	0.0 j	0.0 j

[1]D = Drench and F = Foliar spray

[2]Average number out of 3 inoculated sites per leaf

[3]Average lesion area (mm^2) per 3 inoculation sites per leaf

[4]Numbers followed by the same letter are not significantly different, P=0.05, Duncan's Multiple Range Test

The results of these experiments indicate that a number of fungicides have the potential to control symptom development on conifer shoots and rhododendron leaves inoculated with *P. ramorum*. They also indicate that a number of fungicides that are effective in controlling other types of *Phytophthora* diseases provide limited or no protection against *P. ramorum* on the hosts we tested. Although a number of foliar-applied treatments were very effective in preventing symptom development, it is important to recognize that these treatments were applied under optimal conditions to ensure complete coverage of the developing conifer shoots and rhododendron leaves. It is unlikely that such complete coverage would occur in many production systems where large numbers of plants are grown in close proximity to each other, thus making it very difficult to thoroughly cover susceptible host tissues. In general, fungicides that were applied as drenches were not very effective. None of the drench treatments were effective on the conifers and the Subdue MAXX was the only effective drench treatment on the rhododendrons. It is unclear if some of these products would have worked better if there had been a longer interval between application of the fungicide and inoculation of the plant.

A better understanding of the residual activity of fungicides in prolonging disease control is needed, particularly on hosts such as rhododendron where tissue is susceptible to infection for extended periods of time. Trials that are currently in progress on rhododendron leaves indicate that the residual activity of single applications of some fungicides, such as Ranman, can persist for at least 12 weeks. The isolations we did from symptomatic tissues indicate that fungicide residues on the tissue did not adversely affect our ability to isolate *P. ramorum* (data not shown). Symptomatic tissues were not tested with ELISA or PCR, so it is unclear if any of the fungicide residues might adversely affect the ability to detect the presence of the pathogen using these techniques.

We are also conducting additional experiments to determine what effect fungicide treatments have on symptom development when they are applied to asymptomatic, infected rhododendron leaves. Many of the fungicides that were effective in these trials are contact types of materials that have no systemic activity. While systemic fungicides may have the potential to inhibit symptom development when applied to asymptomatic, infected foliage, it is unclear what if any, effect contact fungicides would have on symptom development. The results of this research will provide a better understanding of the risk that specific fungicides pose in masking symptom development on rhododendron leaves.

Acknowledgments

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Spatial and Temporal Aspects of Tylosis Formation in Tanoak Inoculated With *Phytophthora ramorum*[1]

Brad Collins[2] and Jennifer Parke[2]

Abstract

Phytophthora ramorum is an oomycete pathogen that causes sudden oak death in several species of Fagaceae including tanoak (*Lithocarpus densiflorus*). Symptoms on tanoak include stem cankers and crown death. Stem infection was thought to be restricted to bark and cambium, but has recently been shown to include sapwood.

Woody tissue colonized by fungal pathogens often has a greater abundance of tyloses than non-infected tissue, and tyloses have been interpreted as a host defense response to infection. In this study, we are investigating the spatial and temporal development of tyloses in tanoak logs and living trees inoculated with *P. ramorum*.

In a preliminary study, 30 logs were freshly cut from disease-free tanoak trees in southern Oregon. Half the logs were inoculated at cambium depth with *P. ramorum,* and half were inoculated with a sterile agar plug (wounded controls). At 2, 4, and 7 weeks, sapwood tissue samples were fixed in FAA and hand-sectioned for microscopy. Vessel diameters, the frequency of tylosis occurrence, the presence of hyphae, and the total vessel area occluded by tyloses were recorded. *P. ramorum* appeared to induce tylosis formation in tanoak sapwood by four weeks after inoculation. The spread of hyphae was more rapid than the formation of tyloses, indicating that tyloses may not be an effective defense response in limiting the growth of the pathogen. The relatively slow tylosis formation within tanoak sapwood in response to *P. ramorum* infection may provide insight as to why tanoak is so susceptible to this pathogen.

An unexpected finding was the reduction in the frequency of tylosis occurrence in inoculated logs from four to seven weeks. As the lesions developed, tylosis frequency increased in the xylem vessels. However, tyloses seem to become less frequent within the lesion near the point of inoculation and more frequent near the margin of the lesion. The reduction in tyloses may be due to hyphae within the lesion secreting enzymes that degrade the tylosis cell wall or elicitins that induce a hypersensitive response.

A field study is underway to examine spatial and temporal aspects of *P. ramorum* infection in relation to tylosis formation and specific conductivity of sapwood in living trees. Tanoak trees in California were inoculated with the pathogen in May 2006 and tissue samples were collected in September 2006. Additional samples will be collected in 2007 to track development of the infection and host response.

Key words: Sudden oak death, xylem, host response, stem canker.

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Microbial- and Isothiocyanate-Mediated Control of *Phytophthora* and *Pythium* Species[1]

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Abstract

Plant pathogens of the oomycete lineage share common susceptibilities to many biotic and abiotic stresses. We are investigating the potential of antagonistic bacteria, isothiocyanates, and mycophagous amoebae to control diseases caused by *Phytophthora* spp., including the etiologic agent of sudden oak death, *Phytophthora ramorum* (Rizzo and others 2005), and *Pythium* spp., which cause seedling root rotting diseases across the plant kingdom (Hendrix and Campbell 1973).

Antagonistic bacteria. Inoculation of plant growth media with surfactant-producing bacteria is an established method for preventing oomycete diseases in hydroponic cultures. For instance, infection of water hyacinth by *Pythium ultimum* can be controlled by *Pseudomonas fluorescens* strain SS101, which releases a cyclic lipopeptide surfactant that lyses oomycete zoospores (deSouza and others 2003). We sought to determine whether *P. fluorescens* SS101 treatment could be effective in non-hydroponic systems by investigating its capacity to control root infection by soil-borne *Pythium* spp. and leaf infection by *P. ramorum*.

Cells of strain SS101 and the surfactant-deficient mutant strain 17.18 were inoculated into orchard soil, with concomitant addition of soy flour to stimulate amplification of resident *Pythium* spp. populations. Twelve wheat seeds were sown per treatment. After 24 days seedlings were harvested and 10 1 cm root segments per seedling assayed for infection by *Pythium* spp. Observed infection frequencies of 0.83 percent and 1.7 percent in the SS101- and 17.18 treatments, respectively, were both significantly lower ($P < 0.001$) than the 26.7 percent infection frequency in the nontreated control, implying that the surfactant is not necessary for disease control. These results are consistent with our recent findings of protection against *Pythium* spp. root infection conferred by another surfactant-deficient mutant of strain SS101.[5]

Although we observed that pretreatment with strain SS101 can dramatically lower the incidence *P. ramorum* zoospore-mediated infection of detached bay and rhododendron leaves, the pretreatment was not effective at reducing disease incidence in field trials, most likely due to the diminution of bacterial inoculum on the leaf surface resulting from precipitation events.

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[5] Mazzola, M.; Zhao, X.; Cohen, M.F.; Raaijmakers, J.M. 2007. Cyclic lipopeptide surfactant production by *Pseudomonas fluorescens* SS101 is not required for suppression of complex *Pythium* spp. populations. Phytopathology. 97: 97(10):1348–1355.

***Brassica* spp. seed meals.** Reapportionment of resident soil microbial populations following amendment with *Brassica napus* seed meals can convert a disease-conducive soil into one that suppresses fungal infection of new plantings (Cohen and others 2005; Mazzola and others 2001). The effect appears to result primarily from activation of plant systemic resistance and not direct antagonism of the fungal pathogens (Cohen and Mazzola 2006). If low glucosinolate content seed meal (<30 μmol g^{-1}) is utilized, a post amendment incubation period of several weeks is required in order to permit amplified *Pythium* spp. populations to decline in virulence potential (Cohen and Mazzola 2006). However, many *Brassica* spp. seed meals are high in glucosinolates that upon hydrolysis release isothiocyanates, some of which are highly toxic to oomycetes (Manici and others 1997). We sought to assess the ability of a high-glucosinolate *Brassica juncea* seed meal to inhibit amplification of *Pythium* spp. and, thus, obviate the need for a long post-amendment fallow period.

Seed meal of *B. juncea* cv. Pacific Gold contains high levels (303 μmol g^{-1}) of sinigrin, a glucosinolate that releases allyl-isothiocyanate upon contact with moisture. Incorporation of Pacific Gold seed meal into soil at 0.5 percent (vol/vol) resulted in dramatic long-term reductions in culturable oomycetes and prevented *Pythium* spp. infection of apple seedlings planted into the treated soil (Table 1), consistent with published results (Mazzola and others 2007).

Table 1—*Pythium* spp. population densities in soils and infection frequencies of Gala apple seedling roots

		CV soil		WVC-A soil	
Week[1]	Treatment	cfu/g DWsoil (x 10^2)	Percent Root infection[2]	cfu/g DWsoil (x 10^2)	Percent Root infection[2]
1	Control	0.38 ± 0.24[b]	7.5 ± 2.5[b]	0.80 ± 0.16[a]	11.0 ± 4.8[b]
	Seed meal	n.d.[a]	0.5 ± 0.5[b]	0.24 ± 0.15[a]	n.d.[a]
4	Control	4.0 ± 0.3[c]	27.5 ± 5.3[c]	0.54 ± 0.15[a]	40.5 ± 5.7[c]
	Seed meal	0.44 ± 0.33[b]	n.d.[a]	n.d.[b]	n.d.[a]

[1]Time after incorporation of 0.5 percent (vol/vol) *B. juncea* var. Pacific Gold seed meal amendment into soil.
[2]Root infection frequency values (means ± SE) within a column followed by the same letter are not significantly different. n.d., not detectable.

To determine the ability of *B. juncea* Pacific Gold seed meal to suppress *Capsicum annum* (Bell pepper) seed infection by *Phytophthora capsici*, 3-L samples of garden soil were subjected to various treatments and divided into duplicate plots that were each planted with 25 seeds. Germination frequency after four weeks was 68 percent in unmodified garden soil compared to 6.7 percent in the same soil infested with *P. capsici*. Amendment of *P. capsici*-infested soil with Pacific Gold seed meal immediately prior to planting resulted in a higher germination frequency (46 percent) than did amendment with low-glucosinolate content *B. napus* seed meal (10 percent), or ground *Azolla filiculoides* (12 percent), which does not contain glucosinolates.

Amoebae. Mycophagous amoebae, which consume both fungi and oomycetes, are thought to contribute to the disease suppressiveness of some soils. Amoebae are known to exist on leaves but their role in the phyllosphere habitat is not well characterized (Rodriquez-Zaragoza 1994). From *P. ramorum*-infected leaf lesions of California bay laurel (*Umbellaria californica*), we have isolated several strains of amoebae that are capable of subsisting on cultured *P. ramorum*, one of which, ANN04-395, displays apparent feeding on sporangia (fig. 1). Strain ANN04-395 has been separated from the original host by culture on heat-killed bacteria

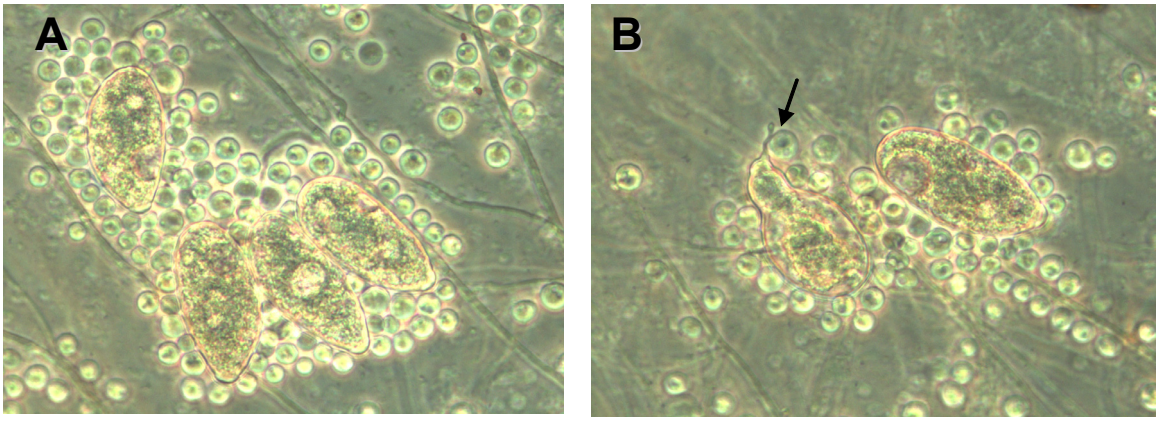

Figure 1—Amoeba strain ANN04-395 associated with *Phytophthora ramorum*. Trophozoites and cysts clustered around sporangia and hyphae of *P. ramorum* (A). Putative feeding of a trophozoite (arrow) on an emergent hypha of an apparently aborted sporangium (B).

and subsequently re-established on another strain of *P. ramorum*. Mycophagy by strain ANN04-395 is not specific to *P. ramorum*, as it is also able to feed upon *P. capsici* and an unidentified ascomycete. To assess biocontrol potential, bay leaves will be pre-treated with amoeba suspensions and assayed for susceptibility to *P. ramorum* and production of functional sporangia within infected lesions. Further development of biologically-based treatments may prove valuable for eliminating *P. ramorum* infestations in nurseries and ameliorating disease severity in landscapes.

Key words: Oomycetes, *Brassica* seed meal, mycophagous amoeba, *Pseudomonas fluorescens.*

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Estimated Economic Losses Associated With the Destruction of Plants Owing to *Phytophthora ramorum* Quarantine Efforts in Washington State[1]

N.L. Dart[2] and G.A. Chastagner[2]

Abstract

The number and retail value of plants destroyed in Washington state nurseries due to *Phytophthora ramorum* quarantine efforts was estimated using Emergency Action Notification forms (EANs) issued by the United States Department of Agriculture Animal and Plant Health Inspection Service between 2004 and 2005. Data collected from EANs indicate that during this period 17,266 containerized nursery plants were destroyed at 32 nurseries, worth an estimated $423,043. The mean loss per nursery was estimated at $11,188 in 2004, $11,798 in 2005, and at $13,220 per nursery over the two-year period.

Key words*: Phytophthora ramorum,* sudden oak death, ramorum blight, economics, quarantine.

Introduction

The quarantined plant pathogen *Phytophthora ramorum* Werres, De Cock & Man in't Veld was first detected in a Washington state nursery in the summer of 2003 during a trace forward survey conducted by the Washington State Department of Agriculture (WSDA). Infected plants were detected at a total of two nurseries in 2003 and WSDA nursery inspections detected *P. ramorum* in 25 nurseries in Washington during 2004 (Jennifer Falacy, personal communication). During late 2004, the United States Department of Agriculture-Animal Plant Health Inspection Service (USDA-APHIS) issued an Emergency Federal Order requiring all nurseries that sell host plant materials in Washington, Oregon and California that ship plants interstate enter a compliance agreement and be inspected for *P. ramorum* starting January 10, 2005 (USDA APHIS 2004)). In 2005 16 nurseries tested positive during certification, recertification or post eradication surveys (Jennifer Falacy, personal communication). Nine of these had tested positive for *P. ramorum* in 2004.

When *P. ramorum* is detected in a Washington nursery, WSDA implements the USDA-APHIS mandated Confirmed Nursery Protocol for *P. ramorum* (USDA APHIS 2006). As part of this protocol an Emergency Action Notification form (EAN) is issued to all nurseries that test positive for *P. ramorum*, notifying the nursery management of the required action(s), and the number and species of plants

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that are subject to the action(s). Depending on the specific circumstances, an EAN will require that plants are destroyed or held for 90 days for additional monitoring.

The economic impact that *P. ramorum* has had on Washington state nurseries is unknown. Although it is difficult to obtain reliable information on losses associated with the disruption of sales and some USDA-APHIS required mitigation actions, information on the number, size, and species of containerized nursery plants destroyed in Washington during 2004 and 2005 is included on EAN documentation. Using EANs and information provided by WSDA, our objective was to estimate the losses experienced by Washington state nurseries due to plant destruction relating to *P. ramorum* mitigation efforts during 2004 and 2005.

Estimating Number and Retail Value of Plants Destroyed Using EANs

Copies of EANs issued in Washington in 2004 and 2005 were obtained from USDA-APHIS. Each nursery was assigned an alpha-numeric code (WA-1 through WA-32) to allow reference to specific nurseries while protecting their identity. The number, species, variety and size of plants destroyed were entered into spreadsheets and sorted by year, nursery code, and species. We calculated the total and mean number of plants destroyed per nursery, as well as the number of plants that were destroyed by species over the 2 year period.

The retail dollar value of the plants that were destroyed was estimated using recommended retail values obtained from a Washington landscape consultant based on size, species, and variety. This information was cross referenced with list prices online and in the field at several retail nurseries in western Washington to confirm the accuracy of the prices in the market place. These values were then used to estimate the total value of the destroyed plants at all nurseries during 2004 and 2005 and the mean loss per nursery.

Numbers and Retail Value of Destroyed Plants

EAN forms indicated that containerized nursery plants were destroyed at 32 different retail nursery sites in Washington in an effort to eradicate *P. ramorum*. EANs documented plant destruction at 22 nurseries in 2004, and 15 nurseries in 2005. The documents indicated that 5 nurseries destroyed plants two consecutive years due to repeat detections by WSDA. Based on WSDA information and EAN documentation, 17,266 containerized nursery plants were destroyed in Washington State between 2004 and 2005. Of these plants, 12,000 were destroyed in 2004 and 5,266 were destroyed in 2005. The mean number of plants destroyed per nursery was 545 in 2004, 341 in 2005, and 540 over the two-year period.

The most commonly destroyed genera of containerized nursery stock in 2004 and 2005 included *Rhododendron* (89 percent), *Calluna* (4 percent), and *Camellia* (4 percent). The total retail value of plants destroyed over the two-year period was estimated at $423,043. The total retail value of plants destroyed in 2004 and 2005 were estimated at $246,144, and $176,899, respectively. The mean loss per retail nursery was estimated at $11,188 in 2004, $11,798 in 2005, and at $13,220 per

nursery over the 2 year period. Five of the 32 retail nurseries that were issued EANs requiring plant destruction accounted for 94 percent of the total estimated value of plants destroyed in 2004 and 2005. These included WA-1 (30 percent), WA-6 (53 percent), WA-8 (2 percent), WA-15 (2 percent), and WA-20 (7 percent). Of these five nurseries, WA-6 was the only one that tested positive in both 2004 and 2005.

Other Costs Associated With *P. ramorum* Quarantine Efforts

We have not attempted to estimate other costs to nurseries associated with implementing the USDA-APHIS confirmed nursery protocol such as labor, fees for burning or burial of plants in a landfill, potential soil and/or water mitigation treatments, as well as the lost opportunity cost associated with placing plants on a minimum 90 day hold for further monitoring. The owners of a nursery (WA-14) that had to destroy 109 plants between 2004 and 2005, accounting for roughly 1 percent of the total retail value of the losses experienced in Washington reported during a phone interview that in addition to the value of the destroyed plants, they spent $30,000 for labor, disposal fees, and mitigation measures at their nursery (undisclosed nursery owner, personal communication). This suggests that the economic impacts of *P. ramorum* on Washington state nurseries are much greater than just the value of the plants that are destroyed.

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Effects of Environmental Variables on the Survival of *Phytophthora ramorum* in Bay Laurel Leaves[1]

M.V. DiLeo,[2] R.M. Bostock,[2] and D.M. Rizzo[2]

Abstract

Bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.) is the primary reservoir host of *Phytophthora ramorum* Werres, De Cock & Man in't Veld in coastal California woodlands. Non-lethal foliar lesions on bay laurel trees support the majority of pathogen sporulation during the winter wet season and appear to provide the primary means of surviving through California's arid Mediterranean summers. Previous experiments have shown that the proportion of symptomatic bay leaves from which *P. ramorum* can be successfully isolated decreases during the summer. In addition, isolation of *P. ramorum* from bay laurel leaves was less at the end of the dry season within mixed evergreen forests when compared to redwood-tanoak forests. These experiments also suggested a positive correlation between bay laurel stem water potential and summer isolation frequency from leaves. A more thorough understanding of the environmental and physiological constraints on the summer survival of *P. ramorum* will assist in the development of SOD risk assessments.

A field study was conducted to investigate environmental and phenotypic correlates of isolation success from bay laurel leaves in the summers of 2005 and 2006. In 2005, 10 trees were monitored at both a mixed-evergreen forest (Fairfield Osborne Preserve) and a redwood-tanoak forest (Jack London State Historical Park). These two sites are approximately 5 km apart on opposite sides of Sonoma Mountain. An additional 31 trees were added in 2006 at 10 sites located in the Sonoma and Mayacmas mountain ranges. Temperature, relative humidity, and vapor pressure deficit data were obtained from weather stations and data loggers placed at each site. Midday stem and leaf water potential was measured with a portable pressure bomb. Both asymptomatic and symptomatic leaves were photographed and analyzed for leaf size and shape, lesion size, and for percent lesion coverage. Survival was measured by isolating from 10 symptomatic leaves from each tree at each timepoint. Isolates from each site were compared for aggressiveness on leaves taken from a single bay laurel tree. Susceptibility of bay trees found at each site were compared by inoculating asymptomatic leaves with either a cocktail of regional isolates, or a single commonly-used lab strain of *P. ramorum*.

The proportion of symptomatic bay laurel leaves from which *P. ramorum* could be successfully isolated fell during both summers. The final proportion of successful isolations varied significantly between sites and years, although the relative differences between sites appeared constant. Large amounts of variation were found within sites that could not be explained by environmental differences. This is likely due to heterogeneous resistance to *P. ramorum* within the bay laurel population. Leaves taken from different trees displayed different resistances to lab inoculations. Leaves removed from bay laurel trees in the late summer appeared to be more resistant to inoculation than those removed in other seasons. This may be due to a direct induced resistance to *P. ramorum* or to indirect resistance mediated by phenological responses to summer environmental conditions. Isolation success

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was associated with phenotypic traits such as leaf size in addition to canopy exposure and midday stem and leaf water potential. Overall it appears that the survival of *P. ramorum* in bay laurel leaves is affected by both a heterogeneously resistant bay laurel population and environmental variables. The survival of *P. ramorum* within bay leaves may be affected by the light interception of individual leaves. The apparent death of the mycelium could be due to direct physical stresses of irradiance such as low water potential, or to indirect physiological changes, which are being tested in lab, growth chamber, and future field experiments.

Key words: *Phytophthora ramorum, Umbellularia californica*, sudden oak death, survival, climate.

Identification of Control Agents and Factors Affecting Pathogenicity of *Phytophthora ramorum*[1]

Marianne Elliott,[2] Simon F. Shamoun,[2] Grace Sumampong,[2] Delano James,[3] Stephan C. Briere,[4] Saad Masri,[3] and Aniko Varga[3]

Abstract

A collection of 67 isolates of *Phytophthora ramorum* from the United States (U.S.), European Union (EU), and Canada was screened using differences in phenotypic traits (pathogenicity, growth rate at several temperatures, and sensitivity/resistance to metalaxyl, dimethomorph, and streptomycin) and for presence of cytoplasmic elements (dsRNA and plasmids). Results of these tests showed a high level of variation among *P. ramorum* isolates. Isolates which differed by +/- two standard deviations from the mean are being examined for presence of dsRNA viruses and DNA plasmids. Plasmids were extracted from some of these isolates and are being characterized. To date, no dsRNA viruses have been isolated.

Key words: *Phytophthora ramorum,* plasmid, dsRNA virus, cytoplasmic elements, pathogenicity.

Introduction

Several hosts for *Phytophthora ramorum* are present in forested and urban areas in Canada. These are primarily foliar hosts that can serve as potential reservoirs for *P. ramorum* inoculum. Establishment of *P. ramorum* on these hosts creates the risk of disease spread to more susceptible hosts in other locations, especially through the nursery trade. In 2004 the Canadian Food Inspection Agency (CFIA) confirmed the presence of *P. ramorum* in imported plants found at a number of retail garden centres in the Vancouver area. These sites tested negative in 2005 but became positive when tested again in September 2006. This was the only incident where *P. ramorum* was detected in Canada. The potential impacts of *P. ramorum* establishment in Canada are estimated to include direct and indirect losses to the horticulture industry and could jeopardize Canada's export trade for rhododendrons, which is valued at over $5 million annually.

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A collaborative project between the Canadian Forest Service – Pacific Forestry Centre and CFIA was initiated to further understand *P. ramorum* pathogenicity and explore potential control measures. The objectives of this research are 1) to examine a large collection of *P. ramorum* isolates from the U.S., Canada, and Europe and screen for the presence of dsRNA viruses, plasmids, and other cytoplasmic elements that affect pathogenicity and 2) screen and test the efficacy of chemical fungicides and biocontrol agents *in vitro* and on *P. ramorum* infected leaves of several plant hosts commonly found in British Columbia nurseries, landscapes, and forests. Preliminary results from the first objective are presented here.

Methods

Phenotypic Characters

Pathogenicity to detached *Rhododendron* leaves, growth rates at 2, 20, and 28 °C, and sensitivity to the fungicides Acrobat (dimethomorph) (0.3 ppm), Subdue Maxx (metalaxyl) (0.2 ppm), and streptomycin (110 ppm) were used to screen a collection of 67 *P. ramorum* isolates representing North American forests and nurseries and European nurseries. Isolates which differed by +/- two standard deviations from the mean values are being examined further for presence of dsRNA and plasmids.

dsRNA and Plasmid Isolation From Various P. ramorum *Isolates*

Mycelial suspensions were prepared by inoculating 500 ml 20 percent V8 (1 percent $CaCO_3$ w/v) with 15-20 mycelial plugs and grown for two weeks at 20-22 °C. Mycelia were harvested by gravity flow filtration and tissues were frozen and stored at -80 °C prior to extraction. To screen for dsRNA, Morris and Dodd's (1979) protocol using CF11 as well as modified protocols from Tooley and others (1989) and Newhouse and others (1992) are being tested. Protocols for plasmid extraction were adapted from Boeke and others (1985), which involves suspension in a sorbitol:EDTA:mercaptoethanol buffer, enzymatic digestion with zymolase (lyticase), and purification with HiPure Plasmid DNA purification kit (Invitrogen).

Results and Discussion

Phenotypic Characters

High variability in pathogenicity was observed among isolates, especially in those from Washington (WA) nurseries (fig. 1). Isolates from Europe (EU), Canadian (CDN) nurseries, and some from California (CA) nurseries were the most aggressive while those from CA forests were the least aggressive. A similar level of variability among isolates was seen in other phenotypic characters (Table 1) but none of these were correlated with pathogenicity (data not shown). Canadian nursery isolates showed low phenotypic variability, suggesting they may belong to the same clone.

Preliminary screening of isolates based on pathogenicity to *Rhododendron* and growth rates at various temperatures has shown great variability among both European and North American isolates. Isolates exhibiting high, moderate, and low pathogenicity are being examined for dsRNA viruses and plasmids. These will be compared to isolates behaving more within the normal range. If dsRNA viruses or

plasmids are detected that are associated with pathogenicity, RT-PCR methods will be developed to screen large numbers of isolates for these elements. In addition, controlled experiments will be conducted to elucidate the biological significance and the role of plasmids and dsRNA viruses in *P. ramorum* and their effects on pathogenicity.

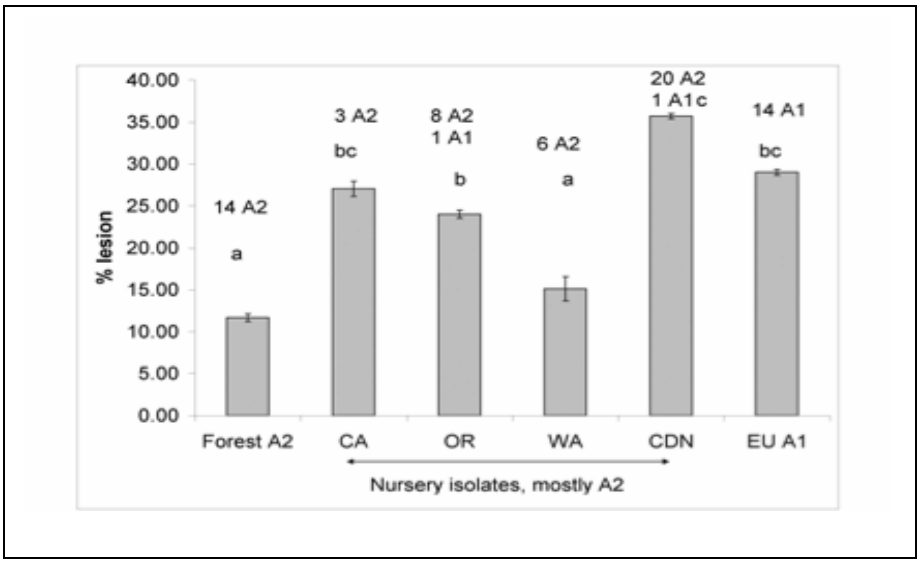

Figure 1.

Table 1—Further information about selected *P. ramorum* isolates. These isolates are being examined for dsRNA and plasmid DNA. Some isolates behaving within the normal range are included for comparison. Heat intolerant – slow growth at 28°C; cold intolerant – slow growth at 2°C.

Isolate number	Strain number	Origin	MT	Characters
0002	MSOD03-0002	Canada	A1	Heat intolerant
5041	Pr-102	OR, USA	A2	Heat intolerant, dimethomorph sensitive
5044	1033.1	OR, USA	A2	Streptomycin, dimethomorph sensitive
5047	4284	OR, USA	A2	Streptomycin resistant
5052	03-156-6	OR, USA	A2	Aggressive
5058	wsda4175	WA, USA	A2	Weakly pathogenic, cold intolerant, fast growing at 20°C
5060	wsda964	WA, USA	A2	Cold intolerant
5061	wsda1839	WA, USA	A2	Weakly pathogenic, cold intolerant, fast growing at 20°C
5063	Wsda3765	WA, USA	A2	Dimethomorph resistant
5064	Pr 0-4	CA, USA	A2	Streptomycin resistant, cold intolerant, fast growing at 20°C
5067	Pr 106	CA, USA	A2	Cold intolerant, heat intolerant
5073	RHCC-23	CA, USA	A2	Aggressive
5074	RHCC-4	CA, USA	A2	Dimethomorph resistant
5078	P1363	UK	A1	Metalaxyl resistant
5079	P1357	UK	A1	Dimethomorph sensitive
5084	CSL2266	Germany	A1	normal
5091	CSL1659	Germany	A1	Metalaxyl resistant
16207	SOD05-16207	Canada	A2	normal
16391	SOD05-16391	Canada	A2	normal
17017	SOD05-17017	Canada	A2	normal
18753	SOD05-18753	Canada	A2	normal

dsRNA and Plasmid Isolation From Various P. ramorum *Isolates*

To date, only the Canadian isolates have been screened for dsRNA. No bands nor viral-associated sequence data has been obtained. Work is ongoing to screen isolates for dsRNA using various protocols. Putative plasmids were found in 4 of five Canadian isolates tested as well as in five other isolates (1 from Germany, 1 from the United Kingdom, and 3 from the U.S.). The approximate size of the putative plasmid is between 14-20kb and the size appears to be variable between isolates. Putative plasmids were found in both A1 and A2 mating types. Work is ongoing to generate sequence information from the plasmid DNA as well as determining the exact size of the plasmids and performing restriction profiling. Further work will examine the relationship of plasmids to virulence and pathogenicity.

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In Vitro Testing of Biological Control Agents on A1 and A2 Isolates of *Phytophthora ramorum*[1]

Marianne Elliott[2] and Simon Shamoun[2]

Abstract

Biological control products were tested *in vitro* with six isolates of *Phytophthora ramorum*. These isolates were geographically diverse and were selected based on their pathogenicity to detached Rhododendron leaves. In addition to five commercially available biocontrol products, nine species of *Trichoderma* were tested. The *in vitro* tests included dual culture methods and detached *Rhododendron* leaf assays. Best results were obtained with the actinomycete *Streptomyces lydicus* (Actinovate®) in both culture and detached leaf tests. There were differences among *P. ramorum* isolates in their sensitivity to the biocontrol agents tested.

Key words: *Phytophthora ramorum*, *Trichoderma* spp., *Streptomyces*, biological control.

Introduction

Phytophthora ramorum, the Oomycete causing sudden oak death, is a problem in wildlands and nurseries (Garbelotto 2004). Biological control methods are being sought as an alternative to chemical fungicide use in nurseries and landscape plantings. Several organisms have been demonstrated to be effective against Oomycetes, such as species of the fungus *Trichoderma*, the bacterium *Bacillus*, and the actinomycete *Streptomyces*. Commercially available products containing these biological control agents exist but have not been tested with *P. ramorum*.

The objective of this study was to evaluate fungal and bacterial antagonists to *P. ramorum in vitro* and on detached leaves of *Rhododendron*. In addition, variability in response to biocontrol agents among isolates of *P. ramorum* belonging to different mating types and from North American and European populations was examined.

Methods

Based on preliminary screening of pathogenicity to *Rhododendron* and growth rates at various temperatures, six isolates of *P. ramorum* were selected for screening of biocontrol agents (BCAs) (table 1).

To test the efficacy of biocontrol products in controlling *P. ramorum*, five commercially available biocontrol products were tested *in vitro* (table 2). In addition, several isolates of *Trichoderma, Penicillium*, and bacteria were tested. Antibiotic production was estimated based on the size of the zone of inhibition (Rajkumar and

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others 2005). For products containing *Trichoderma*, mycoparasitic activity was assessed (Goldfarb and others 1989).

Detached leaves of *Rhododendron* were treated with BCAs at manufacturers recommended concentrations in 200 ml sterile distilled (sd) H_2O and incubated 24 hours in a plastic box containing moist sterile vermiculite in the dark at 20°C. The following day leaves were wounded with forceps next to midrib about and a 7mm plug of *P. ramorum* inoculum placed mycelium side down over the wounded area on the abaxial (under) side. Leaves were sprayed with sdH2O and incubated at 20°C in the dark for 14 days. At the end of the incubation period, percent lesion area was measured using a flatbed scanner and the program ASSESS (Lamari 2002). Percent lesion area was compared to controls not treated with the BCAs.

Table 1—Isolates of *Phytophthora ramorum* selected for the biocontrol study

Isolate	Strain Number	Pathogenicity[a]	MT	Origin	Location
5067	Pr 106	6.51	A2	Forest	CA
5073	RHCC-23	31.09	A2	Nursery	CA
5074	RHCC-4	34.28	A2	Nursery	CA
5039	03-74-D12-A	43.59	A1	Nursery	OR
5084	CSL2266, BBA9/95	32.20	A1	Nursery	EU
5086	CSL2268	31.71	A1	Nursery	EU

[a]Percent lesion area on detached *Rhododendron* leaves after 14 days.

Table 2—Biocontrol products

Name	Organism	Company
Serenade	*Bacillus subtilis* QST 713	Agraquest
Plant Helper	*Trichoderma atroviride* CHS 861	Ampac
Actinovate	*Streptomyces lydicus* WYEC 108	Natural Industries Inc
Companion	*Bacillus subtilis* GB03, other *B. subtilis, B. lichenformis, B. megaterium*	Growth Products
Soilgard	*Gliocladium virens* strain GL-21	Certis

Results and Discussion

Preliminary results indicate that preparations containing *Bacillus subtilis* are very effective in controlling *P. ramorum in vitro* (fig. 1), but best results were obtained with Actinovate® (*Streptomyces lydicus* WYEC 108) on leaves. There was great variability among *P. ramorum* isolates in their behavior with biocontrol agents, both *in vitro* and on detached leaves.

Bacterial biocontrol agents are more effective against *P. ramorum* on leaves than *Trichoderma* (fig. 2). *Trichoderma* may be more effective in controlling the soil phase, or perhaps better formulations of *Trichoderma* are needed for foliar application. Plant Helper (*T. atroviride*) performed very well in *in vitro* tests (table 3), but poorly on detached leaves.

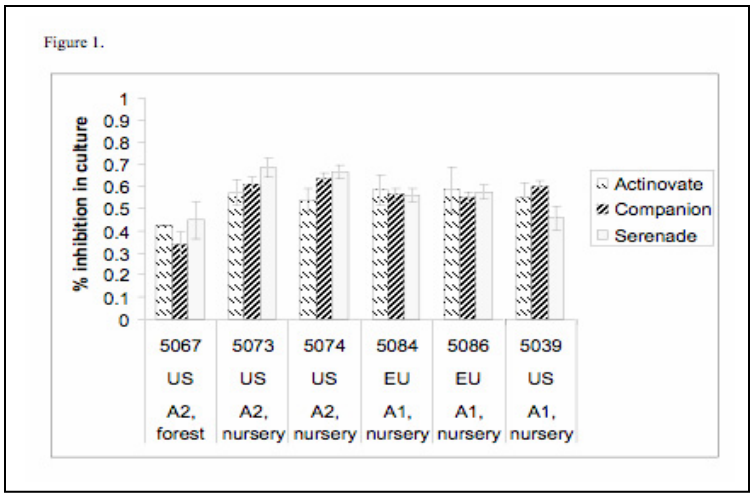

Figure 1—*In vitro* tests of commercial biocontrol agents with six isolates of *P. ramorum*. Percent inhibition of mycelial growth relative to a control plate containing no biocontrol agent is shown.

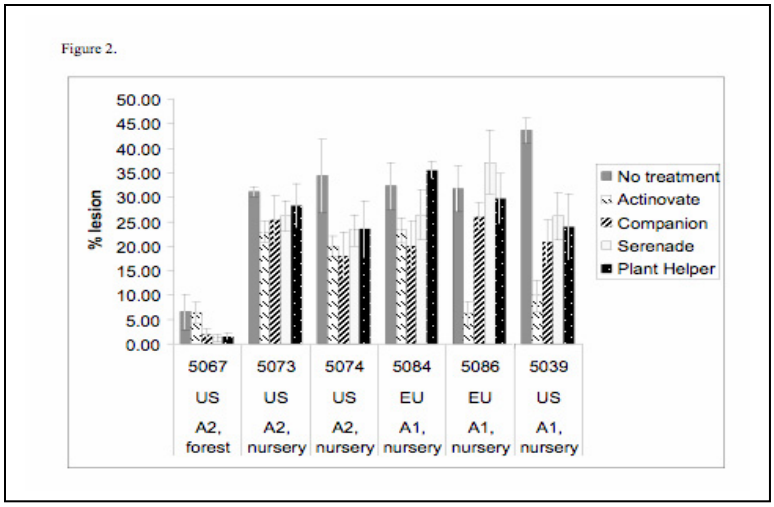

Figure 2—Results of *Rhododendron* leaf tests with four commercial biocontrol agents and an untreated control. Percent lesion area for each treatment is shown. Information about these biocontrol agents can be found in table 2.

Table 3—Percent inhibition of six *P. ramorum* isolates by several *Trichoderma* species

Isolate	Species	A2 5067	A2 5074	A2 5073	A1 5084	A1 5086	A1 5039
PH	*atroviride*	1.00	1.00	1.00	1.00	1.00	1.00
5005	*hamatum*	0.06	0.20	0.26	0.00	0.09	0.00
5006	*hamatum*	0.12	0.61	0.30	0.96	0.96	0.96
5092	*hamatum*	1.00	0.09	0.10	0.25	0.25	0.23
5097	*harzianum*		0.27	0.28		0.22	0.23
5022	*koningii*	0.95	0.57	0.45	1.00	1.00	1.00
5031	*koningii*	1.00	1.00	1.00	1.00	1.00	1.00
5101	*koningii*	0.45	0.07	0.10	0.29	0.29	0.30
5002	*polysporum*	1.00	1.00	1.00	1.00	1.00	1.00
5034	*polysporum*	0.95	0.57	0.37	0.51	0.68	0.89
5093	*pseudokoningii*		0.04	0.00	0.11	0.00	0.02
5094	*saturnisporium*	0.95	0.00	0.02	0.12	0.19	0.00
5095	*virens*	0.00	0.12	0.16	0.00	0.00	0.07
5098	*virens*	0.41	0.06	0.17	0.21	0.26	0.25
5102	*virens*	0.99	0.55	0.57	0.53	0.96	0.48
5020	*viride*	1.00	1.00	1.00	1.00	1.00	1.00
5023	*viride*	1.00	0.91	1.00	1.00	1.00	1.00
5037	*viride*	1.00	1.00	1.00	1.00	1.00	1.00

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New Relationships Among the Sudden Oak Death Pathogen, Bark and Ambrosia Beetles, and Fungi Colonizing Coast Live Oaks[1]

Nadir Erbilgin,[2 5] Brice A. McPherson,[3] Pierluigi Bonello,[4] David L. Wood,[2] and Andrew J. Nelson[2]

Abstract

Sudden oak death (SOD) has had devastating effects on several oak species in many California coastal forests. *Phytophthora ramorum* has been identified as the primary causal agent of sudden oak death. While the pathogen may be capable of killing mature trees, it is likely that in nature opportunistic organisms play significant roles in the decline and death of infected trees. For example, we have found elevated landing rates of bark and ambrosia beetles (Coleoptera: Scolytidae) on mechanically inoculated coast live oaks (*Quercus agrifolia*) in California. The tunneling activity of these beetles in bleeding cankers on *P. ramorum*-infected coast live oaks may accelerate mortality and may contribute to catastrophic failures, even while diseased trees retain asymptomatic canopies. The objective of this study was to determine the role of bark and ambrosia beetle infestation in the introduction and/or stimulation of decay fungi associated with tree mortality and breakage. We inoculated coast live oaks with *P. ramorum* in two forested sites in Marin County in March 2005 and monitored them for signs and symptoms of *P. ramorum* infection. An additional group of asymptomatic trees was felled to allow colonization by bark and ambrosia beetles. In January and July of 2006, we randomly selected and harvested three *P. ramorum*-inoculated trees and three asymptomatic trees from each of the sites. Trees selected for fungal culturing were in the following categories: (1) Asymptomatic trees; (2) Live symptomatic trees exhibiting only bleeding without obvious beetle attacks; (3) Live symptomatic trees exhibiting bleeding with beetle attacks; 4. Dead symptomatic trees with beetle attacks; (5) Dead asymptomatic trees without beetle attacks; (6) Dead asymptomatic trees with beetle attacks. Trees were cut a minimum of 30 cm below the point of inoculation, generating bolts approximately 70 cm long. Each bolt was cut into 15 cm thick disks. Wood samples (5 to 10 mm wide, four per disk) were collected along cross-sectional transects from the upper surface of each disk and divided into four sections. Each section was placed on one of several types of media: potato dextrose agar, malt extract agar and water agar. We separated and purified morphologically distinct fungal colonies (morphotypes) and amplified the internal transcribed spacer (ITS) region of the rDNA operon. Amplicons were sequenced and blasted in GenBank (http://www.ncbi.nlm.nih.gov). The principal taxa isolated from wood samples are described below.

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Pezicula cinnamomea was only isolated from bleeding trees before beetles attacked. Two species, *Botryosphaeria sarmentorum* and an unnamed *Ascomycete* sp. were isolated from infected trees that had not been attacked by beetles, and also from trees that died following beetle infestation. The greatest numbers of fungi were isolated from beetle-colonized, living trees: *Botryosphaeria corticola*, *Geosmithia fassatiae*, *Mucor racemosus*, *Trametes versicolor* and *Truncatella angustata*. Fungi were not isolated from the symptomatic dead trees that died before they had been colonized by beetles. Beetle-attacked, asymptomatic dead trees yielded *B. corticola*, a *Monochaetia* sp. and an *Alternaria* sp. All the fungi identified had ITS values of 97 percent or higher.

Pezicula cinnamomea is generally known as a pathogenic fungus that primarily causes dieback disease of *Quercus* spp., callus rings in *Fagus sylvatica* in Europe and has also been found in *Prunus avium* in Europe and in *Prunus* sp. in Japan. *Botryosphaeria corticola* causes cankers and dieback in *Quercus* spp., and *B. sarmentorum* has been associated with dieback and canker diseases of *Quercus* spp. and *Ulmus*, *Malus*, *Prunus*, and *Pyrus* spp. in Europe. *Geosmithia fassatiae* is an anamorphic fungus found in association with scolytid bark beetle-colonized *Quercus pubescens* in central Europe. *Mucor racemosus* is a filamentous fungus found in soil, plants, decaying fruits and vegetables, while *Trametes versicolor* (known as the Turkey Tail fungus) is found ubiquitously in temperate to sub-tropical forests throughout the world where it serves as a primary decomposer of hardwoods, including *Quercus* spp. *Truncatella angustata* is known to cause disease on stems of *Ribes*, *Prunus* and *Malus* in England. The unknown ascomycete sp. has been isolated from Scots pine (*Pinus sylvestris*) sapwood, at the root collar or in roots. *Monochaetia* spp. cause cankers on several hardwood tree species. *Alternaria* spp. cause serious twig diseases on several hardwood trees, including apples.

These experiments will be repeated in 2007. Our work has revealed the presence of several fungal species commonly associated with disease and decay of hardwood species and appears to be a promising approach in our attempts to fully characterize fungal communities associated with the SOD syndrome. The greatest species diversity was found in infected trees after bark and ambrosia beetles had colonized the sapwood. This study will be expanded and refined so that we can determine the sequence of microorganisms that occur in oaks following infection with *P. ramorum*.

Key words: *Phytophthora ramorum*, sudden oak death, bark and ambrosia beetles, decay fungi, coast live oak.

Summer Survival of *Phytophthora ramorum* in California Bay Laurel Leaves[1]

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Abstract

Sudden oak death manifests as non-lethal foliar lesions on bay laurel (*Umbellularia californica*), which support sporulation and survival of *Phytophthora ramorum* in forest ecosystems. Infected bay laurel leaves are more likely to abscise than uninfected leaves, resulting in an accumulation of inoculum at the forest floor. The pathogen survives the dry summers in a proportion of attached bay leaves, but the histology of colonization during the survival phase and the propagules responsible for survival are unknown. This study focuses on summer pathogen survival associated with bay laurel in redwood-tanoak and mixed-evergreen forests with specific objectives including: i) detection of *P. ramorum* in leaf litter and soils throughout summer, ii) quantification of chlamydospores on and within attached symptomatic leaves, and in fresh and aged litter, iii) determination of chlamydospore germination, and, iv) assessment of pathogen survival within litter and canopy leaves, addressing the location of viable inoculum within foliar tissues.

Ten trees were tagged for repetitive sampling in four redwood-tanoak and four mixed-evergreen forests. Sampling was conducted in May and August 2006. To determine pathogen presence in leaf litter and soil, three soil samples and 20 symptomatic litter leaves were collected and independently bulked from each tree. Samples were then baited for *P. ramorum* with rhododendron leaves. Chlamydospore populations on surfaces of attached leaves, and fresh and aged litter were determined by scrubbing individual leaves with a moistened toothbrush and filtering the resulting suspension through 35 uM nylon mesh. Chlamydospores were then counted under a dissecting microscope and a subsample of chlamydospores was placed on selective medium to observe germination potential. To evaluate chlamydospore production within tissue, leaves were cleared with KOH and then observed with light microscopy. Pathogen survival and colonization was determined by subdividing symptomatic tissue from each leaf for detection by PCR, culture, and microscopy. Furthermore, leaf petioles and midribs were sampled and similarly subdivided for pathogen detection.

P. ramorum was baited from 60 to 90 percent of soil samples at all sites in May 2006, but was undetectable by August. The pathogen was never baited from the bulk leaf litter samples and never isolated from lesions in aged litter; however, sporadic isolation recovery was observed in fresh litter. Pathogen isolation from attached leaves ranged from 40 to 100 percent at each site in May and declined to a range of 0 to 40 percent in August, with higher isolation

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recovery observed in redwood-tanoak forests than in mixed-evergreen forests. Additionally, chlamydospore populations on attached leaf surfaces were higher in redwood-tanoak than in mixed-evergreen forests in both May and August 2006, but no chlamydospore germination was observed, regardless of the origin of the spore. Populations of chlamydospores were present, but highly variable in leaf litter.

Detectable inoculum declined over the duration of the summer with lower foliar survival observed in mixed-evergreen forests than in redwood-tanoak forests. Bay laurel leaves in redwood-tanoak forests supported abundant surface chlamydospore production, but the mechanisms inciting enhanced spore production and the role of these propagules in the survival of *P. ramorum* and epidemiology of sudden oak death is yet unknown.

Key words: *Phytophthora ramorum*, *Umbellularia californica*, survival.

Suppression of *Phytophthora ramorum* in Aluminum-Amended Peatmoss[1]

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Abstract

Phytophthora ramorum, the pathogen responsible for sudden oak death, also impacts the ornamental nursery industry, enhancing the potential for long-distance pathogen transmission in asymptomatic roots or in infested potting media. Soilborne populations of another nursery pathogen, *Phytophthora parasitica*, are suppressed by aluminum (Al)-amended peat, a system where Al activity is regulated by the formation of stable surface complexes with the organic matrix. Organic matter serves as an Al sink, regulating Al activity at a lower level than that predicted by the solubility of Al-bearing minerals and ameliorating phytotoxicity of the metal in soil.

The overall goal of this work was to determine the efficacy of Al-amended peat for suppression of *P. ramorum*. This study addressed i) the effects of Al-amended peat on chlamydospore and sporangium production, ii) the fitness of zoospores produced in Al-amended media, and iii) the influence of Al amendments on resident microbial activity in peat. Limed peat was amended with $Al_2(SO_4)_3$ solutions adjusted to pH 4 or 6 at either 0.0158 or 0.0079 g Al g^{-1} peat. Amended peat was placed in Büchner funnels maintained at –2.5 kPa matric potential and then infested with *P. ramorum* by placing colonized rhododendron leaf disks in each funnel. To determine chlamydospore production, leaf disks were recovered after 7 days, cleared in 1 M KOH, and spores were enumerated under the light microscope. Sporangium production was indirectly assessed by flooding funnels to release zoospores, and estimating propagule density with a soil assay on selective medium. Soil assay plates were then incubated in the dark for five days, and 10 colonies were randomly selected from each treatment to quantify chlamydospore production in colonies arising from zoospores that were formed in amended peat. The microbial activity in Al-amended peat was assessed over a three week period by measuring the rate of CO_2 evolution g^{-1} peat.

All Al-amended peat treatments reduced chlamydospore production by >50 percent compared to the unamended control. Similarly, all Al-amendments reduced pathogen populations, with some treatments resulting in >90 percent reduction in propagule density following flooding. Colonies arising from zoospores formed in Al-amended peat produced at least 50 percent fewer chlamydospores than those that were formed in the unamended control. This suggests that viable zoospores formed in the amended peat have reduced fitness as measured by numbers of chlamydospores produced in the subsequent generation. The initial exchangeable Al levels ranged from 0.33 to 125 μmol Al g^{-1} and the pH ranged from 4.5 to 7.5. The highest exchangeable Al level and lowest pH were observed in the peat amended with the pH 4 solution at a rate of 0.0158 g Al g^{-1}. Conversely, the highest pH was observed in peat amended with the pH 6 solution at a rate of 0.0079 g Al g^{-1}, but no exchangeable Al was detected in this treatment, suggesting the absence of plant-available Al. Furthermore, this treatment also had a negligible effect on microbial activity, whereas the treatment associated

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with the highest exchangeable Al level suppressed microbial activity. After 2 weeks, however, no difference in microbial activity was observed among treatments.

Al-amended peat suppressed asexual reproduction of *P. ramorum* with low levels of exchangeable Al. In fact, pathogen suppression was even observed in treatments with no detectable exchangeable Al, suggesting that the stable surface complexes between monomeric Al species and acid functional groups on the organic matrix are suppressive to *P. ramorum*. Further studies are necessary to determine the mechanism of Al-mediated pathogen suppression and to assess its application in a disease management program.

Key words: *Phytophthora ramorum, Umbellularia californica*, sudden oak death, survival, climate.

Molecular Evolution of an Avirulence Homolog (Avh) Gene Subfamily in *Phytophthora ramorum* [1]

Erica M. Goss,[2] Caroline M. Press,[2] and Niklaus J. Grünwald[2]

Abstract

Pathogen effectors can serve a virulence function on behalf of the pathogen or trigger a rapid defense response in resistant hosts. Sequencing of the *Phytophthora ramorum* genome and subsequent analysis identified a diverse superfamily of approximately 350 genes that are homologous to the four known avirulence genes in plant pathogenic oomycetes and share with them two protein motifs (RxLR and dEER). These have been termed Avh (avirulence homolog) genes. While as a whole the genes in this superfamily exhibit modest sequence similarity, small groups of closely related genes can be identified. We have investigated the molecular evolution of one such group of seven Avh genes. Microarray data suggests that four of these genes are expressed in isolate Pr-102 whose genome was sequenced. We sequenced the full coding region (approximately 400 bp) and flanking noncoding regions of each gene in the three clonal lineages of *P. ramorum*. The number of polymorphic sites within *P. ramorum* genes ranges from 0 to 35, suggesting different evolutionary pressures among genes. Analysis indicates that these genes contain both codons under purifying selection (e.g. in the signal peptide and RxLR and dEER motifs) and under positive selection. We have also been able to obtain the sequence of homologous Avh genes in the sister taxa *P. hibernalis*, *P. lateralis*, and *P. foliorum* allowing for examination of the evolution of these genes across species.

Key words: *Phytophthora ramorum*, *Phytophthora lateralis*, *Phytophthora hibernalis* effector, RXLR motif.

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Effect of Flooding on Root and Foliar Disease Severity on *Rhododendron* Caused by *Phytophthora ramorum*[1]

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Abstract

It is generally thought that extensive periods of flooding can predispose plants to infection by *Phytophthora* pathogens. We evaluated the effect of 0, 1, 3, and 7 days of flooding before infection of *Rhododendron* plants through either wound inoculation of leaves or infestation of the potting mix using two hybrid cultivars 'Catawbiense Boursault' and 'Minnetonka'. Foliage was inoculated with zoospores and potting mix was infested with both zoospores and mycelial agar plugs. Lesion area was quantified using digital imaging. Flooding had no effect on lesion area of foliar infections. Sporangia were retrieved from infected leaves after 10 days of incubation under 20°C ambient containment growth chamber conditions. Root rot developed after about 4 weeks on most plants where the potting mix was infested. Above ground symptoms of potting mix inoculated plants included wilting, yellow or red discoloration, and at times development of lesions similar to those observed in foliar inoculations.

Key words: *Phytophthora ramorum*, flooding, *Rhododendron*.

Introduction

Phytophthora ramorum is a recently emerged oomycete plant pathogen causing a range of diseases including sudden oak death, ramorum shoot dieback and ramorum blight (Rizzo and others 2005; Werres and others 2001). *Phytophthora ramorum* attacks several nursery crops including *Rhododendron*. It is generally thought that extensive periods of flooding predispose plants to infection by *Phytophthora* pathogens (Erwin and Ribeiro 1996). We evaluated the effect of 0, 1, 3, and 7 days of flooding before infection of *Rhododendron* plants through either wound inoculation of leaves or infestation of the potting mix using two hybrid cultivars 'Boursault' and 'Minnetonka'.

Materials and Methods

Two independent experiments were conducted following a randomized complete block design with five replications. Treatments in both experiments were factorial

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and included flooding (0, 1, 3, 7 days), inoculation (non-inoculated control, foliar and potting mix inoculation), and two hybrid *Rhododendron* cultivars (*ponticum* x unknown 'Minnetonka' and *catawbiense* 'Boursault'). Foliage was inoculated with zoospores (40 µl of 5 x 10^4 sporangia per ml pipetted onto cotton set in cryovial caps clipped onto wounded leaves) and potting mix was infested with both zoospores and mycelial agar plugs. Leaf lesion area was quantified using digital image analysis using ASSESS software (American Phytopathological Society, St. Paul, MN). After obtaining digital images, lesions were cut out and placed in 15ml centrifuge tubes with 3 ml of 0.1 percent tween in distilled water. Tubes were vortexed for 30 seconds and leaves were removed. Tubes were centrifuged for 5 minutes at 2500 rpm. Sporangia were counted using a hemocytometer. Infection of roots was confirmed by plating root parts on PARP selective medium followed by microscopic analysis of cultures to determine that *P. ramorum* infected roots. Experiments were conducted in an APHIS approved containment growth chamber held at 14 hour days and constant 20° C temperature. Experiments were analyzed using PROC MIXED in SAS 9.1 for Windows (Cary, NC).

Results and Discussion

Flooding or the interaction between flooding and cultivar had no effect on foliar infections (tables 1, 2). It is possible that flooding for 7 days was not long enough to stress *Rhododendron* plants. Cultivar significantly affected sporulation and disease severity (percent lesion area) (tables 1, 2).

Table 1—Effect of flooding on sporulation of *P. ramorum* on infected leaves of *Rhododendron*

Experiment	Factor	F value	Prob > F
1	Block	1.07	0.392
	Flooding	1.66	0.198
	Cultivar	6.00	**0.021**
	Cultivar × flooding	0.53	0.669
2	Block	0.66	0.627
	Flooding	0.66	0.582
	Cultivar	5.94	**0.021**
	Cultivar × flooding	0.81	0.499

Table 2—Effect of flooding on disease severity determined as percent lesion area on infected *Rhododendron* leaves

Experiment	Factor	F value	Prob > F
1	Block	1.63	0.195
	Flooding	1.32	0.288
	Cultivar	3.03	0.093
	Cultivar × flooding	1.72	0.185
2	Block	1.88	0.142
	Flooding	2.1	0.123
	Cultivar	42.3	**<0.001**
	Cultivar × flooding	0.16	0.923

Flooding or cultivar had no effect on infection of plants through roots ($P > 0.05$). Plants infected through inoculation of potting mix developed disease. Root rot developed after about 4 weeks on most plants where the potting mix was infested. Above ground symptoms of potting mix inoculated plants included wilting, yellow or red discoloration, and at times development of lesions similar to those observed in foliar inoculations. Plating of root segments onto selective medium confirmed infection of plants with *P. ramorum*.

Plants get infected and foliage gets blighted through infested potting mix. These results indicate that *P. ramorum* sporulates readily on *Rhododendron* leaves and has potential for a potting media phase in which it can infect roots. However, further investigation is needed to demonstrate that *P. ramorum* can survive in potting mix and can complete the disease cycle in potting mix.

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Mapping Hardwood Mortality for the Early Detection of *P. ramorum*: an Assessment of Aerial Surveys and Object-Oriented Image Analysis[1]

Erik Haunreiter,[2] Zhanfeng Liu,[3] Jeff Mai,[4] Zachary Heath,[4] and Lisa Fischer[4]

Abstract

Effective monitoring and identification of areas of hardwood mortality is a critical component in the management of sudden oak death (SOD). From 2001 to 2005, aerial surveys covering 13.5 million acres in California were conducted to map and monitor hardwood mortality for the early detection of *Phytophthora ramorum*, the pathogen responsible for SOD. To assess the spatial accuracy of the aerial detection survey (ADS) program data, we used a mortality stem map generated from aerial photo interpretation (Meentemeyer and others 2007) within the Big Sur ecoregion of California. Although results suggest that the aerial surveys may be under-representing the extent of hardwood mortality in the study area, the ADS program has been successful in detecting infestations of *P. ramorum* in California. An additional analysis explored the use of object-oriented classification for mapping hardwood mortality. Results of this analysis indicate that object-oriented image analysis has the potential for mapping hardwood mortality and can complement the ADS program.

Key words: *Phytophthora ramorum*, sudden oak death, aerial surveys, mortality, object-oriented image analysis.

Aerial Detection Surveys (ADS) and Aerial Photo Interpretation

Hardwood mortality was mapped from fixed-wing aircraft using a digital sketch mapping system. From 2001 to 2005, within the Big Sur ecoregion, a total of 9,550 ha with hardwood mortality were mapped. Using high resolution (0.33 m) digital aerial photographs, Meentemeyer and others (2007) digitized point locations of all visible dead trees within a 794 km^2 area of the Big Sur ecoregion. The digitized point locations include all mortality up to 2005.

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Aerial Survey Data Assessment

Using the digitized point locations of dead trees as a reference data set, we assessed the spatial accuracy of the aerial survey data within the Big Sur ecoregion study area. Results suggest that the aerial surveys in this region are under-representing the extent of hardwood mortality in the Big Sur ecoregion (52 percent of the digitized points fell within the ADS polygons) (fig. 1).

Figure 1—Detail showing ADS mapped mortality polygons and Meentemeyer and others (2007) digitized point locations of dead trees.

Object-Oriented Image Analysis

Using the same set of digital aerial photos, four small areas of a single photo were selected to test the ability of object-oriented image analysis to identify single dead trees on the landscape. An initial test run was performed using eCognition 4.3 Professional. Using the digitized point locations of dead trees from Meentemeyer and others (2007), the object-oriented classified mortality map was spatially overlaid onto the digitized point map to calculate the percent overlap (fig. 2). The object oriented classification captured 71 percent of the mapped stems in the sample area.

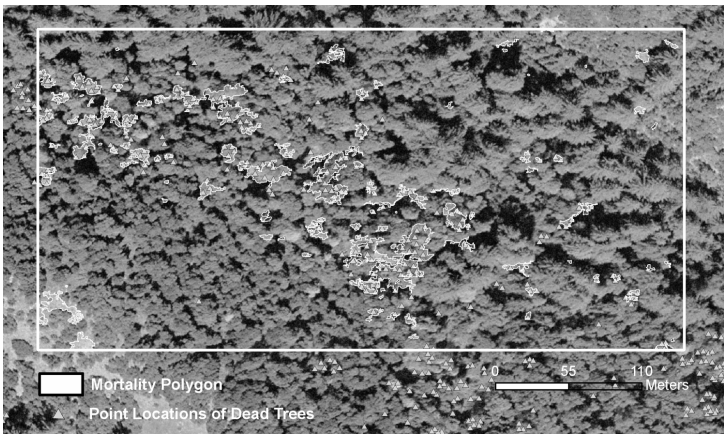

Figure 2—Detail showing object-oriented mortality polygons and digitized point locations of dead trees.

Conclusions

The primary advantage of the aerial detection surveys is that they cover a wide area in a short period of time for a relatively low cost. A disadvantage of the ADS data is a loss of resolution in the data as a result of the scale of the program.

Object-oriented classification has the potential to complement the ADS program, especially in analyzing areas of concern identified by the aerial surveys. Further work is required to explore applying object-oriented image analysis with field data for validation.

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Correlating *Phytophthora ramorum* Infection Rate and Lesion Expansion in Tanoak[1]

Katherine Hayden,[2] Heather Rickard,[2] and Matteo Garbelotto[2]

Abstract

To date, resistance to *Phytophthora ramorum* in its most susceptible hosts has most commonly been quantified by lesion growth, after wounding or non-wounding inoculations via mycelia or high concentrations of zoospores. However, even highly susceptible hosts may not always become infected when they are exposed to a pathogen under ecologically realistic conditions. Therefore, resistance quantified by lesion growth may obscure an important pathway for pathogen resistance: the initial infection process. Disease resistance in plants may be characterized as either the host's ability to prevent infection by a pathogen (measured by a presence or absence of pathogen), or the plant's capacity to restrict pathogen growth within its tissues (measured by lesion extent). Here we describe an experiment designed to determine 1) if the two measurements are correlated in the *Lithocarpus densiflorus* (tanoak)-*P. ramorum* system, and 2) if lesion development measured in leaf tissue corresponds with lesions that develop in tanoak twigs, as they often appear in wild settings.

We previously surveyed tanoak populations to determine whether lesion area in a detached-leaf assay varied significantly among individual trees and sites. We returned to two sites with highly significantly different means, and collected branches from 14 previously assayed trees with a wide range of apparent disease resistance. From each tree, 10 branches were inoculated with a 10^4 spores/ml zoospore solution dropped onto the leaf axel, and incubated at either 10°C or 20°C, roughly the range of daytime temperatures to be expected during the rainy season on the California and Oregon coasts. After 2 weeks, pieces from the twigs and leaves were plated on selective media and monitored for *P. ramorum* growth.

Two trees (those previously ranked highest in susceptibility) yielded positive isolations of *P. ramorum* from sterile water sham inoculations. These were presumed to be already infected with the pathogen, and were therefore excluded from all analyses. The remaining trees were analyzed for infection frequencies, as well as the number of positive isolations from each twig, a measure of lesion extent. Across both sites, we found no statistically significant relationship between infection frequency in the twigs and susceptibility as measured by a detached leaf assay (logistic regression, $P = 0.24$). The pathogen was detected significantly more often after incubation at 20°C than at 10°C ($P = 0.035$).

The mean area of leaf lesions in the previous assay ($\log_e$-transformed for analysis) had a significant effect on the number of positive isolations per twig (analysis of variance,

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P = 0.007), reflecting an association between lesion spread in leaves and lesion spread in twigs. Incubation temperature also had a positive effect on the number of positive isolations ($P < 0.0001$).

We conclude that lesion spread in twigs is correlated to lesion spread in leaves. Further, this measure of resistance is durable over time, as nearly 2 years passed between the leaf and twig inoculations. However, lesion spread in leaves and twigs in tanoak is not correlated with zoospore infection rates.

Key words: *Phytophthora ramorum*, tanoak, resistance.

Geographical Distribution of *Phytophthora ramorum* in Norway[1]

María- Luz Herrero,[2] Brita Toppe,[2] and Trond Rafoss[2]

Abstract

In November 2002, *Phytophthora ramorum* was detected for the first time in Norway. It was isolated from *Rhododendron catawbiense* imported earlier the same year. After the first detection, the Norwegian Food Safety Authority has carried out surveys from 2003 to 2006. The surveys were first directed to nurseries and garden centres. Most of the positive findings were on *Rhododendron* spp., but one sample of *Pieris japonica*, one of *Kalmia* sp., one of *Syringa* sp. and one of *Viburnum* sp. were also positive. In 2005 and 2006, *P. ramorum* was isolated from well-established viburnum and rhododendron plants in private gardens, parks and public greens. Infections were detected on well-established plants from nine different outdoor sites. While the positive samples from nurseries were spread over the country, the positives found on well-established plants were concentrated in and around the cities of Bergen and Stavanger on the southwestern coast of Norway. This part of the country has cool summers, mild winters and more than 2000 mm of annual precipitation. In 2006, *P. ramorum* was found in private gardens in the eastern part of the country on young recently introduced plants. *P. ramorum* was found in 12 import shipments, mainly on rhododendron, but also on a viburnum sample.

Key words: *Phytophthora ramorum*, Norway, survey.

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Antimicrobial Activity of Extracts and Select Compounds in the Heartwood of Seven Western Conifers Toward *Phytophthora ramorum* [1]

Daniel K. Manter,[2] Rick G. Kelsey, [3] and Joseph J. Karchesy [4]

Abstract

Previously, we demonstrated that wood chips, essential oil, and four individual compounds from Alaska yellow-cedar (*Chamaecyparis nootkatensis* [D. Don] Spach) heartwood strongly inhibit the germination of *Phytophthora ramorum* (Werres, de Cock, Man int Veld) zoospores or sporangia, and reduce hyphal growth in culture (Manter and others 2006). Essential oils from heartwood of incense cedar (*Calocedrus decurrens* [Torr.] Florin), western redcedar (*Thuja plicata* Donn ex D. Don), Port-Orford-cedar (*Chamaecyparis lawsoniana* [A. Murr.] Parl.), and western juniper (*Juniperus occidentalis* Hook.*)* were also tested and found to be equally active, but not investigated further until now. The objectives of this study were to: 1) test the procedure described by Kuhajek and others (2003) as a bioassay technique for *P. ramorum*; 2) use this bioassay with other individual compounds from yellow-cedar heartwood that were not previously tested for bioactivity toward *P. ramorum*; and 3) bioassay extracts and compounds from the heartwood of the other conifers with antimicrobial essential oils and compare their *P. ramorum* activity to the yellow-cedar constituents.

Bulk heartwood samples were gathered from one tree of the five species above, plus Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) and redwood (*Sequoia sempervirens* [D. Don] Endl.). Air dried chips were ground (20 mesh), extracted with ethyl acetate (triplicate subsamples), filtered and bioassayed. In addition, 14 compounds known to occur in the heartwood of these seven conifers were selected for testing individually (hinokitiol, thymoquinone, nootkatin, nootkatol, carvacrol, valencene-11, 12-diol, α-terpineol, valencene-13-ol, taxifolin, (+)-β-cedrene, (-)-thujopsene, (+)-cedrol, δ-cadinene, methylcarvacrol). They were all commercially available, or previously isolated in our laboratory, and soluble in ethyl acetate.

Zoospores were obtained from one *P. ramorum* isolate (A1 Oregon source) after growing on clarified V8 agar (6.6 percent) for two weeks. One isolate was considered adequate based on previous results (Manter and others 2006). Bioassays of *P. ramorum* growth were conducted in a 96-well microtiter format by measuring optical density (OD, 650 nm) at 0, 16, 24, 48 and 72 h using a microplate reader (Kuhajek and others 2003). Antimicrobial activity of all test compounds was analyzed by adding zoospore solution (50 µl, 10^4 zoospores ml^{-1}) and V8

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broth (100 µl, 6.6 percent) amended with an extract, or individual test compound to each of six replicate wells. Heartwood extracts were tested at eight concentrations from 0 - 10000 ppm (v/v). Individual compounds were tested at eight concentrations from 0 - 1000 ppm (v/v). All experimental plates also included six replicate sterility control wells (100 µl of V8 broth; 50 µl dH_2O), and any plates with growth in these wells were discarded. All experimental plates were replicated three times and their average OD values used for growth analysis. For each concentration of extract, or compound bioassayed, a sigmoidal curve was fitted to the OD growth values to obtain an estimate of $time_{1/2}$, or time (h) it takes to reach the OD mid-point between its minimum and maximum value. Antimicrobial activity of each test compound was then determined by fitting a sigmoidal regression curve to the $time_{1/2}$ vs. log_{10} concentration (ppm) plot and calculating the EC_{50} from this curve.

After completing the bioassays, chemical constituents in heartwood extracts of incense cedar, western red cedar, Port-Orford-cedar, and western juniper were identified by gas chromatography-mass spectrometry (GC-MS). Constituents in yellow-cedar extracts were identified recently by GC-MS and were recognizable from their relative retention times. Concentrations of compounds in the extracts were determined by adding an internal standard and analyzing them by GC with an FID detector.

To determine whether chips of red cedar heartwood can impact *P. ramorum* under natural conditions we conducted a field test on the Rush Creek Open Space Preserve, at Novato, California. Bulk samples of red cedar (same tree sampled for lab bioassays) and redwood (commercial boards) chips were prepared in January 2006, and enclosed (140 g dry wt equivalent) in nylon mesh bags (approx. 20 x 20 cm). Redwood was selected as a control because it was inactive toward *P. ramorum* in culture bioassays (see below). On March 1, 2006 ten California bay laurel (*Umbellularia californica* [Hook. & Arn.] Nutt.), trees with leaves symptomatic of *P. ramorum* infection were selected to serve as blocks in the preserve. Two plots (1 x 1 m) were located beneath each tree where the canopy had the most symptomatic leaves. Six bags of red cedar chips were randomly positioned within one plot and six bags of redwood in the other plot. Bags were placed on top of the litter and secured with long wire pins in the soil. After about two months (May 2) the litter and soil (1.4 cm dia. x 3.0 cm deep) beneath three randomly chosen bags were sampled on each plot. Sampling was repeated for the remaining three chip bags after four months (June 29). Total genomic DNA was extracted from each litter or soil sample using plant or soil DNA kits and the manufacturer's protocol. DNA from the litter samples was further purified by phenol: chloroform:isoamyl alcohol (25:24:1) extraction and ethanol precipitation. Amplification of *P. ramorum* DNA was performed using the species-specific nested protocol developed by Hayden and others (2006). The amount of *P. ramorum* DNA present per unit dry weight of soil or litter was calculated from measured Ct values (BioRad, iCycler) and an external standard curve generated from serially diluted *P. ramorum* genomic DNA isolated from pure cultures.

The Kuhajek and others (2003) bioassay procedure was easy to use, allowed many samples to be quickly analyzed, and required small amounts of media and test materials. Disadvantages include the inability to directly test heartwood activity because of interference with light transmittance. Also, it does not distinguish inhibition or disruption of zoospore or sporangia germination from inhibition of hyphal growth. Nevertheless, this photometric method is useful for detecting compounds with antimicrobial activity toward *P. ramorum*.

The heartwood extracts were grouped into three levels of antimicrobial activity: 1) strong (EC_{50} 500-700 ppm) – for incense cedar and western redcedar; 2) moderate (EC_{50} 1500-2100 ppm) – yellow-cedar, western juniper, and Port-Orford-cedar, and 3) weak or no activity (EC_{50} > 10000 ppm) – Douglas-fir and redwood. Since the chemical composition of conifer heartwood can be extremely variable among trees, our ranking of species activities above applies only to the samples used in this study. Confirmation of these species differences will require testing a larger random sample. Four categories of activity were used for the

individual compounds tested: 1) strong (EC_{50} 1-10 ppm) – hinokitiol, thymoquinone; 2) moderate – (EC_{50} 11-100 ppm) nootkatin, nootkatol, carvacrol, valencene-11, 12-diol; 3) weak (EC_{50} 101-1000 ppm) – α-terpineol, valencene-13-ol, taxifolin, β-cedrene, and thujopsene; and 4) no activity (EC_{50} > 1000 ppm) – cedrol, δ-cadinene, and methylcarvacrol. All compounds with strong or moderate antimicrobial activity contained a free hydroxy group, except for thymoquinone. The importance of this functionality is demonstrated by the large difference in activity between carvacrol (EC_{50} 59.8 ppm) and methylcarvacrol (> 1,000 ppm), with a methyl group blocking the hydroxyl.

Major components in the heartwood extracts were distinct for each species, except for a few select compounds. Activities of the heartwood extracts tended to parallel the activities of individual compounds they contained. For example, extracts of incense cedar and western redcedar expressed the strongest antimicrobial activity and they each contained one of the individual compounds with strong activity; thymoquinone in incense cedar and hinokitiol in western redcedar. Yellow-cedar extracts were moderately active and contained numerous compounds that individually were moderate inhibitors (nootkatin, nootkatol, carvacrol, valencene-11, 12-diol, and valencene-13-ol). Western juniper and Port-Orford-cedar extracts also were moderately antimicrobial, but their individual compounds we tested had only weak, or no activity. The chemical constituents in Douglas-fir and redwood extracts were not analyzed further by GC because they lacked activity.

On May 1, two months after placing chips on the forest floor there was no difference between the western redcedar and redwood plots in the amount of *P. ramorum* DNA in litter. However, during the two months between May 1 and June 29 *P. ramorum* DNA on the redwood control plots increased 11.1 times, compared with only 2.6 times on the redcedar plots. At the end of June the redwood control plots had 4.3 times more *P. ramorum* DNA than the western redcedar plots. Soils from redwood and redcedar plots had the same quantities of DNA in May and June. Increases in *P. ramorum* biomass, and DNA, during the spring rains is thought to occur from either spores washing from the surface of infected leaves by rainfall (Davidson and others 2005), or newly germinated chlamydospores in the litter, which give rise to new sporangia and zoospores and a new infection cycle. Redcedar chips were effective in reducing the inoculum potential and future development of new infections.

An integrated management program may be able to utilize bioactive conifer heartwoods in some instances to help limit the proliferation of *P. ramorum* spores in litter and the potential for new infections via splash dispersal (Davidson and others 2005), or long-distance transport to new areas. For example, people living, working, and recreating in areas infested with *P. ramorum* can vector spores on their shoes, bicycle tires, or other items that contact spore contaminated soils or plant tissues (Cushman and Meentemeyer 2006, Davidson and others 2005). Previously we proposed that chips of yellow-cedar might be spread over trails, bike paths, or parking lots used by recreationists to reduce spore movement and distribution from these areas (Manter and others 2006). Our results here suggest heartwood chips from western redcedar, or incense cedar are as active, and possibly more active toward *P. ramorum* than those of yellow-cedar. Both of these species are abundant and readily attainable in large volumes from western forests. Alternatively, some of the most active individual heartwood compounds might be developed into useful products. It may be worthwhile examining the efficacy of foliar spray applications for some of the most active compounds such as hinokitiol and thymoquinone.

Key words: Photometric bioassay, conifer heartwood, antimicrobial activity.

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Evaluation of a Rapid Diagnostic Field Test Kit for Identification of *Phytophthora ramorum*, *P. kernoviae* and Other *Phytophthora* Species at the Point of Inspection[1]

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Abstract

Plant health regulations to prevent the introduction and spread of *Phytophthora ramorum* and *P. kernoviae* require rapid, cost effective diagnostic methods for screening large numbers of plant samples at the time of inspection. Current on-site techniques require expensive equipment, considerable expertise and are not suited for plant health inspectors. Therefore, an extensive evaluation of a commercially available lateral flow device (LFD) for *Phytophthora* species was performed involving four separate trials and 634 samples. The assay proved simple to use, provided results in a few minutes and on every occasion a control line reacted positively confirming the validity of the test. LFD results were compared to those from testing a parallel sample, using laboratory methods (isolation and real-time PCR). The diagnostic sensitivity of the LFD (87.6 percent) compared favourably to the standard laboratory methods although the diagnostic specificity was not as stringent (82.9 percent). There were a small number of false negatives, but for statutory purposes where all positive samples must be identified to species level by laboratory testing, overall efficiency was 95.6 percent as compared to visual assessment of symptoms of between 20-30 percent for *P. ramorum* and *P. kernoviae*. This work demonstrates the value of the lateral flow device for diagnosing *Phytophthora* species at the time of inspection and as a useful primary screen for selecting samples for laboratory testing to determine the species identification.

Key words: *Phytophthora ramorum*, *P. kernoviae*, lateral flow device.

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Materials and Methods

Lateral Flow Device

Phytophthora LFD kits designed to recognise all species of *Phytophthora,* including *P. ramorum* and *P. kernoviae,* were supplied by Forsite Diagnostics Ltd, York, UK. Detailed instructions on LFD use were supplied. In summary, several small pieces of leaf showing symptoms were broken up between the thumb and fingers before placed in a plastic bottle containing 5 small (approximately 3mm) ball bearings and extraction buffer. Pieces of suspected diseased tissue transfer into the extraction bottle. The bottle was shaken vigorously for 60 seconds and then the extract taken up in a small disposable dropper. Two to four drops were placed onto an absorbent pad within the kit and left for at least 2 minutes but no longer than 10 minutes before reading. A single blue line developed to indicate the test kit was working (control line) whilst the development of a second blue (target line) indicated the presence of *Phytophthora* spp. A larger sample from the same part of the plant with identical symptoms was submitted for laboratory testing according to a protocol developed at CSL and now part of the EPPO Diagnostic protocol (Anonymous, 2006).

LFD Sensitivity

The sensitivity of the LFDs was evaluated using naturally infected rhododendron leaves submitted as part of routine plant health surveillance and previously tested as positive for *P. ramorum* by isolation and real-time PCR. A small square of necrotic tissue (approx. 12 x 12 mm) was excised from the leaf the wet weight determined. It was then dissected further into smaller portions, the wet weights determined and then tested with an LFD. A similar piece of known healthy leaf tissue (12 x 12 mm) was tested in addition to neat buffer solution. The presence of a test line was visually scored after 5 minutes in addition to quantification of the intensity of the test using an optical reader (Chromatoreader Type 2, Otsuka Electronics Co., Japan). Using the optical reader a negative result is recorded as zero. Lines may be visible at between optical reader values of 4-10, but are easily seen in excess of 15 and may rise to in excess of 100. The experiment was repeated with three unrelated samples.

LFD Specificity

The specificity of the LFD was evaluated using a range of cultures. A small piece of agar (1 cm^2) was excised from the centre of the colony, placed in an extraction bottle, shaken vigorously for 10-15 seconds and then tested with a LFD as described above. Devices were read after 5 minutes and scored visually as either negative or positive.

Comparative Testing

A large number of plant samples submitted by Defra's Plant Health Inspectors were tested using both the LFD and conventional methods as described above. The type and host distribution of samples tested during this trial was representative of material submitted during the UK national survey for *P. ramorum* although the majority of samples tested were rhododendrons.

Results

Sensitivity

A positive reaction was clearly obtained with just a few mg of necrotic rhododendron leaf tissue (equivalent to a few square millimetres) either alone or when mixed with healthy leaf tissue permitting detection in leaf tissue which was less than 1 percent infected by *P. ramorum*.

Specificity

The negative control (agar plug), true fungi (*Alternaria alternata*, *Botrytis cinerea*, *Cylindrocarpon* sp., *Monilinia laxa*, *Pleospora herbarum*, *Trichoderma harzianum*, *Rhizopus* sp.) and isolates of the oomycete *Pythium* also all tested negative. All 13 species of *Phytophthora,* including *P. ramorum* and *P. kernoviae*, tested positive.

Comparative Testing

A total of 634 samples were tested with agreement on 536 occasions (84.5 percent). False positives and negatives were encountered on 70 (11.0 percent) and 28 (4.4 percent) occasions respectively. The diagnostic sensitivity was 87.6 percent and the diagnostic specificity was 82.9 percent (Table 1).

Table 1—Comparison of the Lateral Flow Device with existing diagnostic methods for detection of *Phytophthora* illustrating diagnostic sensitivity ($\frac{A}{A+C}$) and specificity ($\frac{D}{D+B}$)

		Isolation		
		+	-	Total
LFD	+	197 (*A*)	70 (*B*)	267
	-	28 (*C*)	339 (*D*)	367
	Total	225	409	634

Notes

A LFD and comparative both positive;
D LFD and comparative test both negative;
B LFD positive but comparative test negative (false positive);
C LFD negative but comparative test positive (false negative).

Diagnostic sensitivity = 87.60% and Diagnostic specificity = 82.9% .

Discussion

A commercially available lateral flow device for *Phytophthora* (Forsite Diagnostics Ltd, York) identified the presence of *P. ramorum, P. kernoviae* and other *Phytophthora* species on a wide range of plant material as part of plant health inspection and disease management work. The assay was demonstrated to identify a broad range of *Phytophthora* species and did not cross react with other true or lower

fungi. The LFD was shown to be very sensitive and able to detect *P. ramorum* in less than 1 percent infected rhododendron leaf tissue. The assay was simple to use, provided results in 3-5 minutes and on every occasion a control line appeared confirming the validity of the test. LFD results were compared to those from testing a parallel, but not always identical, sample using well-established laboratory methods. For *P. ramorum* this has been extensively evaluated with a diagnostic specificity of 99.3 percent and diagnostic sensitivity of 92.3 percent when isolation was compared to direct real time PCR for a large number of samples (Hughes and others 2006).

These trials demonstrate that LFDs offer a useful decision support tool for the detection of *Phytophthora* spp. at the point of inspection. For statutory purposes, as positive LFD results require laboratory testing to determine the species of *Phytophthora*, the presence of false positives is overcome. Therefore, in this study where all LFD positives were submitted for laboratory testing, an overall efficiency of 95.6 percent was achieved which is a substantial improvement on relying on visual assessment alone. The LFD kits for detecting *Phytophthora* spp. cost from £6 per test so are significantly cheaper than laboratory testing. They have been of considerable value in instructing new plant health inspectors in disease recognition, helping to convince growers and land-owners of the need to sample and hold plants. The simplicity and robustness of these kits makes them ideally suited for all skill levels and their size and weight ideal for varying sites and conditions to obtain a rapid assessment of whether *Phytophthora* may be present. They have the potential to assist plant health organisations manage their disease campaigns in a new way by helping to optimise and target the use of field inspectors, highly skilled diagnostic staff and centralised laboratory services.

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Comparative Susceptibility of Plants Native to the Appalachian Range of the United States to Inoculation With *Phytophthora ramorum*[1]

R.G. Linderman,[2] Patricia B. de Sá,[3] and E.A. Davis[2]

Abstract

Phytophthora ramorum, cause of sudden oak death of trees or ramorum blight of other plant species, has many hosts. Some geographic regions, such as the Appalachian range of the eastern United States, are considered high risk of becoming infested with the pathogen because known susceptible plants occur there and climatic characteristics appear favorable for infections by this pathogen. We collected foliage of a range of plant species native to Appalachia in Kentucky during two summer seasons, and inoculated the foliage in Oregon with *P. ramorum* to determine relative susceptibility. Some genera, species, and cultivars within species were highly susceptible, while others were moderately susceptible or not susceptible. These results provide a basis for regional surveyors to select target hosts and to generate survey and management practices for nursery and forest areas.

Key words: Appalachia, *Phytophthora ramorum*, sudden oak death, ramorum blight.

Introduction

The discovery of sudden oak death or ramorum blight, caused by the pathogen *Phytophthora ramorum*, on a wide range of trees, shrubs, and ornamental plants in U. S. nurseries, landscapes, and natural ecosystems, has resulted in quarantine regulation of the pathogen to prevent geographic dissemination. However, geographic distribution of the pathogen has occurred, both nationally and internationally, putting new regions and plant ecosystems at risk.

Plants native to the Appalachian range of the eastern U.S. are especially at risk due to the favorable environmental conditions for the disease. Based on the known host list, native and horticultural varieties of rhododendrons, viburnums, mountain laurel, maples and other plants growing in central Appalachia could become infected with *P. ramorum*. Eastern U.S. regions are currently being surveyed in an attempt to detect introductions from other regions, especially in areas surrounding nurseries where disease has been found. However, the relative susceptibility of many of the over-story, mid-story, and under-story plants in those areas is unknown, making field inspections extremely difficult due to the high diversity of plant species therein.

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Some knowledge of the relative susceptibility of eastern native plant species would be extremely useful to inspectors, and could be a basis for management strategies for the nurseries and for eastern forests to reduce the risk of establishment of *P. ramorum*. Thus, our objective was to determine the relative susceptibility to inoculation with *P. ramorum* of a sampling of several important, representative plant species found in the Appalachian range.

Methods

We collected foliage of a range of plant species native to Appalachia in Kentucky and inoculated with *P. ramorum* to determine their relative susceptibility. Leaves were needle-wounded on the upper surface and inoculated with sporangia of a North American A2 mating type, isolate N10 (Linderman and others 2006). After 14 days incubation at 20°C in moist boxes, lesions caused by inoculation, compared to the uninoculated control, were measured using digital photos and ASSESS software (Lamari 2002).

Results and Discussion

Quantitative estimates of lesion sizes (percent of total leaf area with lesions resulting from inoculation) indicated considerable variation in susceptibility to *P. ramorum*. Some species were highly susceptible, while others were moderately susceptible or not susceptible (figs. 1 to 3). Results on understory and mid- to overstory plants indicate the high susceptibility of plants such as black cherry, black walnut, green ash, and the low susceptibility of plants such as some red maples, hickory, sweetgum, and others. While there was considerable variation in reaction to inoculation between plant species, that variation could easily have been due to the time of plant tissue collection, variation in edaphic and other environmental conditions, and even age of plants. These results provide a basis for regional surveyors to select target hosts and to generate survey and management practices for nursery and forest areas.

Fig. 1—Lesions on mid- and over-story plants inoculated with *P. ramorum* N10 sporangia. Top to bottom: spice bush, prairie rose, bladdernut, and alder.

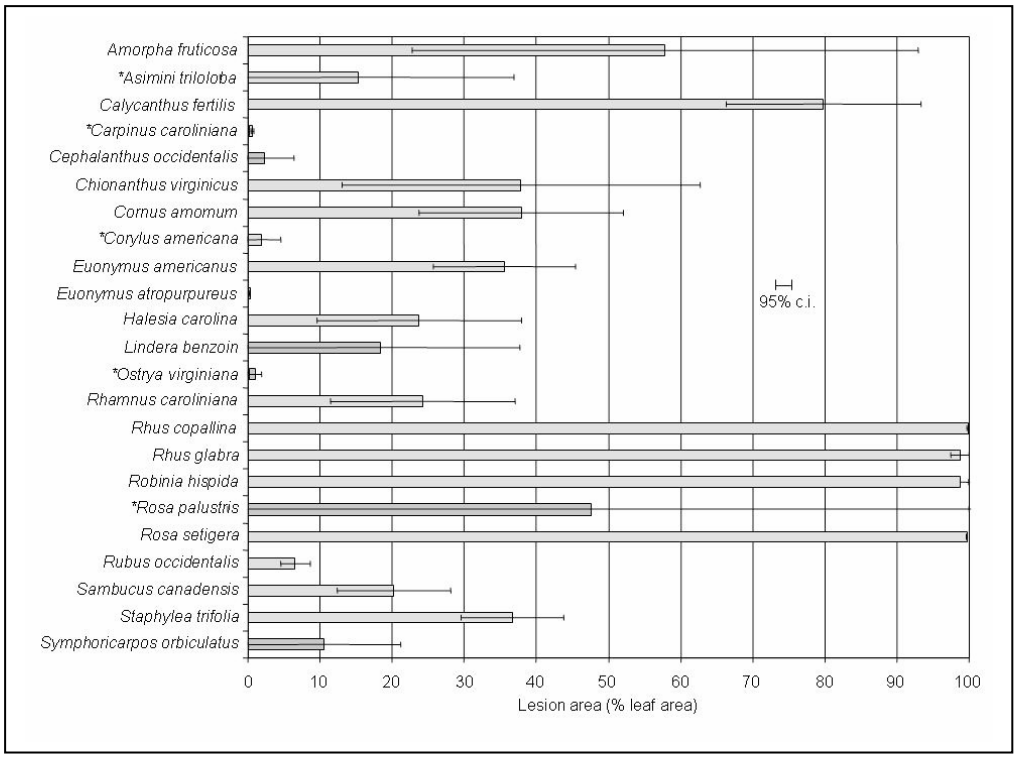

Fig. 2—Lesion areas of understory plants inoculated with *P. ramorum* N10 sporangia.

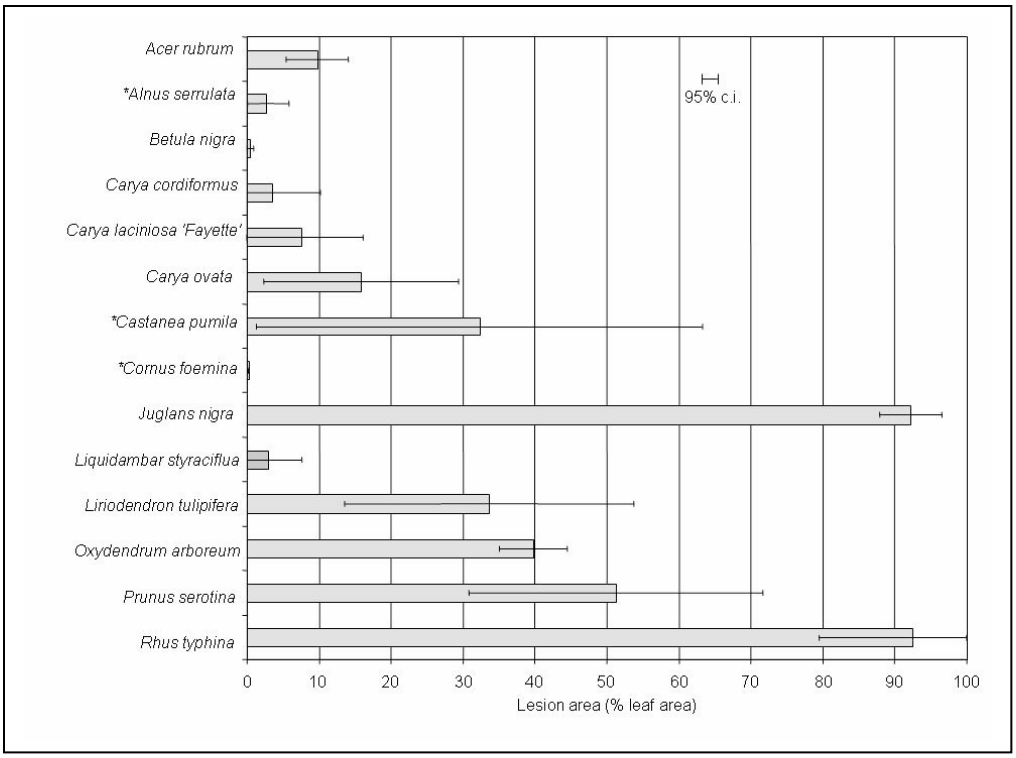

Fig.—3. Lesion areas of mid- and over-story plants inoculated with *P. ramorum* N10 sporangia.

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Pathogenicity Variation in Two West Coast Forest *Phytophthoras, Phytophthora nemorosa* and *P. pseudosyringae*, to Bay Laurel[1]

R.E. Linzer[2] and M. Garbelotto[2]

Abstract

Two recently described pathogenic oomycetes, *Phytophthora nemorosa* and *P. pseudosyringae*, have overlapping host and geographic ranges in California and Oregon forests with *P. ramorum*, causal agent of "sudden oak death" disease. Preliminary genetic evidence indicates *P. nemorosa* and *P. pseudosyringae* may be introduced in this region; their sympatric distribution with *P. ramorum* suggests they may be an interacting factor in *P. ramorum* disease.

The ultimate goal of this project is to characterize the outcome of interaction during infection by *P. ramorum, P. nemorosa* and *P. pseudosyringae* in the epidemiologically important host, bay laurel (*Umbellularia californica*). Experiments will account for variability in host, pathogen and environment, and leaf lesion area in detached bay leaves will be used as the proxy for pathogenicity. Therefore, the proximal goals of this portion of the project are to describe pathogenicity variability and refine methods for leaf infection. Here, we describe four preliminary experiments conducted to select *Phytophthora* isolates for inoculation and to optimize leaf infection methods.

1) In order to identify a subset of *Phytophthora* isolates with sufficient sporangia production for infection and to determine the liquid medium yielding highest sporangial concentration, we screened 15 California and Oregon isolates of each *Phytophthora* species for sporangia formation. All isolates were originally recovered from *U. californica.* Agar disks, 5 mm in diameter, were excised from the margin of *Phytophthora* colonies growing on V8 agar and placed in the bottom of separate wells in 24-well micro-titer plates. Three replicates of each isolate were incubated in each of three liquid media: distilled water and two-percent infusions of soil or *U. californica* leaves in distilled water. Liquid media was added until even with the top surface of each disk, and plates were incubated for five days. Maximal production of sporangia was achieved in the soil-water infusion for all *P. nemorosa* isolates. Though sporulation varied by medium for *P. ramorum* and *P. pseudosyringae*, consistent high sporangial production was also achieved in two-percent soil-water infusion. Thus, we will proceed with the soil-water infusion as the induction medium for subsequent experiments. Isolate sets of each species were reduced to the 10 highest sporulators, excluding isolates with sparse or absent sporangia.

2) Next, we tested for zoospore release from the selected 10 sporangia-producing isolates of each species. Agar plugs from colonies growing on V8 medium were incubated with soil-water

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added up to the plug surface. *P. ramorum* isolates were incubated for three days at 18°C and *P. nemorosa* and *P. pseudosyringae* for five days at 14 and 18°C, respectively. To induce zoospore release, agar plugs and induction medium were incubated at 4°C for 30 minutes then for 45 minutes at room-temperature before zoospore quantification. Though all isolates yielded abundant sporangia, zoospore release was extremely variable. Release was plentiful from all *P. ramorum* and some *P. pseudosyringae* isolates but absent or too low to count in all *P. nemorosa* and the remaining *P. pseudosyringae* isolates.

3) To allow for zoospore release from all isolates, we then tested different induction procedures, comparing the standard *P. ramorum* release parameters, above, to two cycles of temperature shock and a temperature shock cycle of 30 minutes at 4°C followed by a room-temperature recovery time of 2.5 hours. Although the two temperature shocks yielded zoospore numbers indistinguishable from the standard protocol, increasing the recovery time resulted in a sufficient zoospore concentration to undertake subsequent inoculations.

4) To further refine leaf inoculation methods, we used a subset of two isolates of each species and compared two detached-leaf inoculation methods: a zoospore-suspension drop on the abaxial leaf surface vs. dipping leaf tip in a zoospore suspension. As *P. nemorosa* has a lower optimal *in vitro* growth temperature than do *P. ramorum* and *P. pseudosyringae,* we added the effect of environmental variability on infection outcome by undertaking the experiment in parallel at two temperatures: 12 and 18°C. For the abaxial leaf drop method, detached leaves of approximately the same age from a single bay host were infected by dropping 50 µl of a 2 x 10^4 zoospores/mL suspension on the abaxial leaf surface one cm from the tip of the leaf. Alternately, for the leaf dip method, leaves were infected by tip-down immersion in a 50 mL conical tube containing 300 µl of a 2 x 10^4 zoospores/mL suspension and removed form the suspension after 24 hours. Leaves were incubated on 1 cm-gridded racks in partially sealed plastic boxes lined with moist paper towels. Leaves were misted every two days and harvested on the ninth day. Resulting leaf lesion area was quantified using APS Assess. Both inoculation methods resulted in measurable lesions, and lesions developed at both temperatures for five of the six isolates. The trend was toward larger lesions at 18°C for all species, though most differences were not significant. Many drop lesions were significantly larger then lesions resulting from leaf dips. Since the leaf area exposed to inoculum can be more easily standardized using the leaf drop method, we will use this method in subsequent experiments.

Using methods and isolates determined above, we will compare pathogenicity of the 10 isolates of each of the three species identified in step 2) at three temperatures: 12, 18 and 24°C, against leaves of one intermediately susceptible bay laurel host. The range of pathogenicity variability thus identified will be utilized to select isolates for competition experiments.

Key words: *Umbellularia californica*, detached leaf, zoospore, sporangia, inoculation.

Monitoring *Phytophthora ramorum* and *P. kernoviae* in Soil and Rainwater Samples Collected at Two Sites on a Cornish Estate[1]

David Lockley,[2] Judith Turner,[3] Gillian Humphries,[3] and Phil Jennings[3]

Abstract

Soil samples were collected from quadrats marked out below the canopies of two rhododendrons, one infected by *Phytophthora ramorum* (Site 1) and the other (Site 2) infected by *Phytophthora kernoviae* and *P. ramorum.* Rainwater was collected in high-level and low-level traps. Soil and rainwater were sampled at roughly monthly intervals from November 2003 until April 2006, and tested for the presence of *P. ramorum* and *P. kernoviae* at the Central Science Laboratory. The infected rhododendrons were removed in May 2004 (Site 1) and July 2004 (Site 2) after which the quadrats and rain traps were reinstated. *P. ramorum* was initially detected in soil from less than a third of the quadrats at Site 1 and declined to very low levels after removal of the host. All rainwater samples at Site 1 were negative for *P. ramorum*. At Site 2, both *P. ramorum* and *P. kernoviae* were initially readily found in soil samples. After removal of the host, levels of *P. kernoviae* in the soil declined rapidly, but *P. ramorum* was still detected in a relatively high proportion of soil samples for up to 21 months. *P. ramorum* was found in rain traps at Site 2 over the winter of 2006, indicating an influx of wind blown spores. Rhododendron leaf baits buried in the soil at Site 2 frequently became infected by *P. ramorum* for up to 21 months after removal of the infected host.

Key words: *Phytophthora ramorum*, *Phytophthora kernoviae*, soil, rainwater, rhododendron.

Introduction

An extensive outbreak of *Phytophthora ramorum* was discovered in 2003 at a large estate in Cornwall containing one of the world's great collections of magnolias, camellias and rhododendrons, with many specimens originating from China and planted a hundred years ago. Further investigations revealed the presence of a new *Phytophthora* species, later named *P. kernoviae*.

Materials and Methods

Two sites with infected rhododendrons, one with *P. ramorum* (Site 1) and the other with both pathogens (Site 2), were selected in November 2003 for monitoring work. Nineteen 1m^2 quadrats were marked out on the ground below the rhododendron

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canopies at each site and leaf litter and/or soil was sampled from each quadrat at roughly monthly intervals. Rain traps were also set up and sampled monthly. High-level rain traps consisted of a sterile plastic bottle fitted with a 15cm diameter funnel, secured to a post 1m above ground level. Low-level rain traps were introduced later and consisted of a plastic bowl placed on the ground. Four or five clean rhododendron leaves were clamped between two plastic discs and placed in the bowl, which was then covered with fine netting to prevent leaves being blown into the trap.

Samples were tested for the presence of *P. ramorum* and *P. kernoviae* at the Central Science Laboratory, York. Soil samples were placed in a plastic sandwich box and Petris mineral solution added until the soil was just covered. Pieces of fresh, clean rhododendron and magnolia (evergreen) leaves were then floated on the solution. After 7 days the rhododendron and magnolia pieces were removed and rinsed twice in tap water before being plated onto *Phytophthora* selective media (PARPH). After a further 7 days the plate was visually assessed for the growth of any *Phytophthora.* Positive visual assessment was then validated using TaqMan real time PCR. Water samples from high-level rain traps were processed in a similar way to the soil samples, with rhododendron and magnolia leaf pieces floated directly on the water sample.

The rhododendron at Site 1 was removed in May 2004 and the plant at Site 2 in July 2004 after which the quadrats were reinstated and monitoring continued until April 2006. In order to determine whether the recovery of *P. ramorum* from soil samples indicated a risk to susceptible plants growing on the site, soil baits were buried every two or three months after removal of the infected rhododendron at Site 2. The baits, which consisted of cut pieces of rhododendron leaves enclosed in small muslin bags, were buried just beneath the soil surface in a quadrat which had previously yielded positive soil results. The baits were retrieved after a week and the leaf pieces soaked and then rinsed twice before being plated on PARPH and processed as above.

Results and Discussion

Soil contamination by *P. ramorum* was initially relatively low at Site 1 with only 31 percent of quadrats positive in December 2003. After the removal of the infected host, *P. ramorum* was detected in 0 to 10 percent quadrats over the following winter and spring, and then only occasionally in 5 percent samples. *P. ramorum* was never detected in any of the rain traps at Site 1, although *P. kernoviae* was recovered from a low-level trap in November 2005.

P. ramorum and *P. kernoviae* were readily detected in soil from quadrats at Site 2 prior to removal of the host. After removal, *P. ramorum* was still found in a high proportion (up to 63 percent) of quadrats for 21 months, after which time, monitoring was concluded. Recovery of *P. ramorum* was greatest in the winter and spring when soils were wetter, than in the summer. *P. kernoviae* however, declined and was rarely recovered in soil samples after removal of the infected host. No *P. ramorum* was detected in rain water samples from high-level traps before removal of the host, but it was detected in rain collected over the winter of 2006 in both high- and low-level traps, suggesting an influx of wind blown inoculum. Surprisingly, despite

considerable initial foliar infection by *P. kernoviae* at Site 2, the pathogen was not recovered from any of the rain traps.

Soil baits at Site 2 frequently became infected by *P. ramorum*, but never by *P. kernoviae*. Infection was lower in the summer 2005 and greater in the winter and spring 2006. At the final baiting in April 2006, all baits became infected by *P. ramorum*. The presence of *P. ramorum* in soil sampled in 2006 may be due to new inoculum deposited by wind and rain or long term survival of chlamydospores originating from infected leaf litter.

Acknowledgments

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Monitoring *Phytophthora ramorum* in Soil, Leaf Litter, Rain Traps, and Watercourses in an Historical Cornish Garden[1]

David Lockley,[2] Judith Turner,[3] Gillian Humphries,[3] and Phil Jennings[3]

Abstract

Phytophthora ramorum was identified as the cause of a leaf blight on rhododendrons in an historic garden in Cornwall in 2003. A programme of measures was set in place to eradicate the disease from the garden and several sites were selected to monitor the effect of these measures on the recovery of *P. ramorum* from soil, leaf litter, rainwater and watercourses. The results from two monitoring sites are presented plus the results of leaf baiting of the watercourses. After the removal of the infected rhododendron at Site 1 and the application of a composted mulch over the soil surface, *P. ramorum* was no longer detected in the soil samples. At another site (Site 4), which consisted of a clump of several large 'Cornish Red' rhododendrons, removal of the host was not practical, and instead, the lower branches were removed to encourage airflow through the canopy. Although the detection of *P. ramorum* in soil samples has gradually declined at this site, fallen leaves have continued to harbour the pathogen, with some seasonal variation showing lowest recovery during the summer months. Recovery of *P. ramorum* from watercourses was also lowest in the summer and greatest in the spring.

Key words: *Phytophthora ramorum*, rhododendron, soil, rainwater, watercourses.

Introduction

Phytophthora ramorum was first identified as causing infection on a number of rhododendrons in the garden in July 2003. A programme of work was initiated to eradicate the disease without destroying the important historical features of the garden. Several monitoring sites were established to assess the effects of the work on the recovery of *P. ramorum* from soil, leaf litter, rainwater and watercourses.

Materials and Methods

In December 2003, a grid of eight $1m^2$ quadrats was marked out on the ground at a site (1) below a canopy of *Quercus ilex*, camellia and a rhododendron infected by *P. ramorum*. Rain traps were also set up to collect rainwater passing through the canopy. Initially, these were at ground level, but later they were raised to 1m above ground level. Low-level rain traps were reintroduced in October 2005, this time with rhododendron leaf baits included. Soil and/or leaf litter samples were collected from

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each $1m^2$ quadrat at roughly monthly intervals until June 2004 when the infected rhododendron was removed. The number of quadrats was then increased to 12 and sampling continued until February 2005 when a layer of composted mulch, about 10cm deep, was spread over the soil surface. The soil and mulch layer were then sampled separately until April 2006.

A further infested site (4) was identified in May 2004. This consisted of a clump of several 'Cornish Red' hybrid rhododendrons which formed a major feature of the gardens. Grids (four groups of three $1m^2$ quadrats) were marked out beneath the canopy and high-level rain traps were set up at three points. Low-level rain traps were introduced in October 2005, adjacent to the high-level traps. Soil and fallen leaves were sampled from each quadrat at roughly monthly intervals until December 2006. Rainwater was also collected from the rain traps at each site at each visit. The soil, fallen leaves and rainwater samples were examined for the presence of *P. ramorum* at the Central Science Laboratory, York, using techniques described elsewhere (Lockley and others, this volume). Several small pools and some larger ponds were also tested for the presence of *P. ramorum* using rhododendron leaf baits, consisting of small ($1cm^2$) pieces of rhododendron leaf contained in a muslin bag. Baits were left in the water for periods of 7-10 days.

Results and Discussion

P. ramorum was detected in soil/leaf litter at Site 1 mainly during the winter months and in one quadrat in May. Once the composted mulch had been applied, *P. ramorum* was no longer detected in the soil, although it was occasionally found in the mulch. Low-level rain traps at Site 1 were positive for *P. ramorum* in March and April 2004, and between November 2005 and May 2006. High-level rain traps gave positive results in June and December 2005 and again in March 2006.

Isolations from fallen leaves at Site 4 consistently yielded *P. ramorum*. Initially in the summer 2004, only 40 to 50 percent of quadrats were positive, but by November 2004, *P. ramorum* was isolated from 100 percent of samples. Levels of infection decreased in August and September 2005, but recovered again and remained high until May 2006. Samples collected in June and September 2006 showed a marked reduction in infection before levels rose again in December 2006. Despite the high levels of infected leaves recovered from quadrats on the ground, the soil samples (with the exception of a few quadrats) generally failed to yield *P. ramorum*.

Baiting of watercourses generally showed low levels of *P. ramorum* in the summer and winter, but samples taken in the spring and, to a lesser extent, in the autumn, were frequently positive for *P. ramorum*.

Seasonal variations in the recovery of *P. ramorum* have been demonstrated in this garden. The low level of infection found in fallen rhododendron leaves at Site 4 during August and September contrasted with much higher levels found over the rest of the year. Interestingly, these higher levels of detection were not accompanied by obvious increases in foliar symptoms, which were mainly confined to young, soft growth in the spring. This period coincided with the most frequent recovery of the pathogen from watercourses in the gardens.

The measures taken at Site 1 to minimize the disease (removal of the infected rhododendron and application of composted mulch over the soil surface) appear to have been successful. *P. ramorum* has not been recovered from the soil or mulch at that site since December 2005. At site 4, the removal of lower branches in autumn 2004 to allow greater flow of air through the interior of the 'Cornish Red' appears to have had little effect on leaf infection, although soil infection has gradually declined.

The low-level rain traps have been more successful in trapping *P. ramorum* than high-level traps, probably because they are prone to rain splash from the soil surface, but the inclusion of rhododendron leaves in these traps may have contributed towards greater recovery of *P. ramorum*.

Acknowledgments

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Development of *Phytophthora ramorum* Infection and Disease Symptoms on Coast Redwood Seedlings[1]

Sunny Lucas,[2] Jennifer L. Parke,[2] and Yana Valachovic[3]

Abstract

Coast redwood (*Sequoia sempervirens*) is a host for *Phytophthora ramorum* but it is not clear if the pathogen represents a significant disease risk to this tree species. In an on-going field experiment, we are examining the process of infection and the development of symptoms on coast redwood seedlings in naturally infested sites in southern Humboldt County, California. In November 2006, healthy, potted redwood seedlings were placed amid tanoak and bay trees naturally infected with *P. ramorum.* Symptoms and disease incidence are being observed periodically, and every two months, a subset of redwood seedlings is destructively sampled to investigate the location and extent of tissue colonization by *P. ramorum.* To assess inoculum levels to which seedlings are exposed, and to correlate this with disease development, rainwater is being collected for quantification of *P. ramorum* propagules. Results of this study will help evaluate the risk of *P. ramorum* to coast redwood seedlings and inform land managers of the potential for reforestation of infested sites with this species.

Key words: *Phytophthora ramorum*, *Sequoia sempervirens.*

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Photosynthetic Declines Are Induced by *Phytophthora ramorum* Infection and Exposure to Elicitins[1]

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Abstract

Infection of compatible plants by *Phytophthora* spp. often leads to a decline in stomatal conductance and photosynthesis, although the mechanistic basis for such declines is not completely understood. In many cases, declines in leaf gas exchange rates have been linked to losses in water supply capacity associated with root and/or xylem. However, the reductions in gas exchange may not be proportional to changes in hydraulic capacity, and may be observed in non-invaded regions, suggesting the presence of a toxin, or host-derived signal, that is responsible for some of the physiological impairment.

In the current study, we first conducted a series of experiments to determine if toxins secreted by *P. ramorum* are likely contributors to physiological injury in the host by examining the temporal changes in photosynthesis, stomatal conductance, and hydraulic conductivity of *Rhododendron macrophyllum* G. Don (rhododendron) artificially inoculated with *P. ramorum*. Second, we tested the ability of culture filtrates and purified, recombinant *P. ramorum* elicitins (i.e., the major proteins secreted by *P. ramorum* grown *in vitro*) to induce physiological changes in incompatible *Nicotiana tabacum* (tobacco) and compatible tanoak, rhododendron, and *Umbellularia californica* (California bay laurel) host species.

To determine whether toxins secreted from *P. ramorum* contribute to physiological injury in the host, two stems (ca. 2.5 cm dia) from each of 12 three-year-old rhododendron plants were artificially inoculated with a 5 mm dia hyphal plug cut from ca. 2 week-old *P. ramorum* starter cultures (2 percent cornmeal agar) or uninoculated control plates, which was secured under the bark, ca. 15 cm below the lowest leaf, using dH_2O-saturated gauze. The *P. ramorum* isolate used for inoculation was a North American mating type (A2) obtained from infected native plants growing in Curry County, Oregon. On a weekly basis (1-4 weeks after inoculation), A/C_i curves (net CO_2 assimilation over a range of CO_2 concentrations), stem-specific hydraulic conductivity, and stem lesion lengths were monitored.

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Phytophthora ramorum elicitins purified from culture filtrates or obtained from a prokaryotic expression system (pET SUMO expression system, Invitrogen, Carlsbad, CA) were tested for their ability to cause physiological damage when applied to the four host species listed above. Measured responses included H^+ uptake, ethylene production, and chlorophyll fluorescence.

A search of the *P. ramorum* genome project (DOE Joint Genome Project, http://genome.jgi-psf.org/ramorum/) revealed five sequences coding for recognizable elicitin proteins (protein ID: 47381, 47386, 47376, 71636, and 78569). Based on these sequences, two conserved primer sets were designed to amplify full-length elicitin genes. High homology between the gene sequences prevented the design of individual primer sets for all five sequences. Primer sets were as follows: ram-α15'-GAACTTCCGCGCCCTG and 5'-ACAGCGACGCGCACGT) and ram-α2 (5'-ATGCAGTTCGCCGCTCTC and 5'-TACAGCGACGCACACGT). The two primer sets were tested on six different *P. ramorum* isolates, producing two unique elicitin proteins common to all six isolates. Full-length ram- α1 and ram- α2 genes were cloned into the pET SUMO vector, induced with 1 mM IPTG for 6 h at 37°C, and purified by affinity chromatography (ProBond Resin, Invitrogen, Carlsbad, CA). The purity of the recombinant elicitins was verified visually by SDS-PAGE.

All artificial inoculations of rhododendron were successful, resulting in an average lesion length of 6.9 $\pm$ 0.9 cm by the end of the four week study. Reisolation of *P. ramorum* was 100 percent successful from all symptomatic stem tissues, but not from any of the asymptomatic stem or leaf tissues. Physiological changes developed rapidly in leaves of the inoculated stems, despite the lack of visible symptoms in the leaves or petiole. Three weeks after inoculation, when stem lesion lengths were 4.4 $\pm$ 0.6 cm, V_{cmax} (maximum rate of carboxylation limited by the amount, activity, and kinetics of rubisco) was reduced by ca. 21 percent. Additional declines occured during the fourth week, after the development of significant impacts on plant-water-relations.

The functionality of *P. ramorum* infected stems to supply water to host leaves and maintain photosynthetic rates was assessed from K_S (stem-specific hyrdaulic conductivity) and g_s (stomatal conductance) measurements. Four weeks after inoculation, but not before, both measures declined; g_s, a measure of stomatal openness, declined by 36 percent, and K_S, a measure of xylem water supply capacity, declined by 64 percent. A culture filtrate derived elicitin from *P. ramorum* was purified and tested for its ability to influence leaf processes. Similar to the artificial inoculation experiment, the CF-elicitin caused a significant decline of 23.4 percent in photosynthetic capacity and 14.8 percent in the efficiency of open PSII centers (F_v/F_m). Two components often associated with the hypersensitive response (HR), H^+ uptake and ethylene production, were also influenced by elicitin uptake changing by 78.8 and 92.4 percent, respectively.

The two purified, recombinant elicitins (ram-α1 and ram- α2) were tested for biological activity in both compatible and incompatible hosts. Both recombinant elicitins produced a visible hypersensitive response and developed necrotic areas when infiltrated into leaves of the incompatible host, tobacco; however, no macroscopically visible necrosis was observed in any of the three compatible hosts. Independent of the development of visible necrosis, the recombinant elicitins significantly affected a variety of physiological characteristics of all four host species. In all species, exposure to recombinant elicitins caused a decline in the maximum efficiency of PSII centers or F_v/F_m, while enhancing H^+ uptake and ethylene production, relative to the controls. Thus, for all treatment combinations (elicitin and host species) the decline in F_v/F_m was strongly and positively correlated to H+ uptake ($R^2 = 0.801$) and ethylene production ($R^2 = 0.884$). Like the culture filtrate tests, tobacco exhibited the greatest responses, followed by tanoak, myrtle, and rhododendron. For all three measures, ram-α1 triggered significantly greater responses compared to ram-α2, except in rhododendron, and in tanoak F_v/F_m.

While toxins have been suggested to play a role in *Phytophthora* spp. pathosystems, previous efforts to document elicitin toxicity in compatible hosts have met with varying degrees of success. For example, elicitin exposure did not influence stomatal conductance in chestnut (Maurel and others 2004) or net photosynthesis in beech (Fleischmann and others 2005). However, ultrastructural changes in oak (Brummer and others 2002) and varying degrees of necrosis or cell apoptosis have been observed in several Solanaceae plants (Vleeshouwers and others 2000). Based on these observations and those of the current study, a wide range of host responsiveness to elictins is possible. Although the mechanistic basis for the observed photosynthetic declines was not fully explored in this study we hypothesize that it is associated with an incomplete or hypersensitive-like response. In part, this hypothesis is based on the strong correlation between the decline in F_v/F_m and two processes typically associated with HR: H^+ uptake and ethylene production. To date, the vast majority of work with elicitins has focused on their ability to induce the HR and systemic acquired resistance in incompatible hosts such as tobacco (Bonnet and others 1996). Furthermore, both artificial inoculation (Scharte and others 2005) and elicitin exposure (Matsumura and others 2003) result in photosynthetic declines in incompatible hosts. Part of this decline surely arises from the death of functional mesophyll cells during a successful HR. However, Scharte and others (2005) recently showed that a successful HR requires the suppression of photosynthesis, associated with callose depositon and/or sugar accumulation, before HR cell death can be initiated. Thus, it follows that host differences in the degree of the HR response to elicitins (i.e., highest in resistant species) could be the source of the observed photosynthetic declines (i.e. highest in resistant species) in response to elicitin infiltration. Consistent with this hypothesis, Vleeshouwers and others (2000) examined HR cell death in several *Solanum* clones and found a high degree of variation in the timing and degree of HR cell death, which was correlated with resistance to *P. infestans*. Finally, the notion of an effector triggering HR-like processes in both compatible and incompatible hosts is supported by other studies. For example, the NPP1 effector from *Phytophthora* species induces typical HR-associated (ethylene accumulation, callose deposition, and necrosis) and SAR-associated (pathogenesis-related gene accumulation) processes in both compatible and incompatible host species (Fellbrich and others 2002).

In conclusion, we have shown that exogenous application of elicitins results in photosynthetic declines in both compatible and incompatible hosts. The mechanism responsible for the declines is unknown but may be associated with quantitative differences in the timing and degree of a hypersensitive-like response. Previous studies have shown that elicitins are avirulence factors in some nonhosts, such as tobacco (Kamoun 2006). In this study, elicitin sensitivity was inversely related to *P. ramorum* susceptibility (tobacco > tanoak > myrtle > rhododendron). It is unknown if host sensitivity to elicitins directly contribute to quantitative differences in host susceptibility to *P. ramorum*; however, elicitins appear to contribute to virulence by directly reducing the photosynthetic performance of its host.

Key words: Chlorophyll fluorescence, elicitin, photosynthesis, sudden oak death, toxin.

Acknowledgments

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Evaluation of Molecular Markers for *Phytophthora ramorum* Detection and Identification Using a Standardized Library of Isolates [1]

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Abstract

A number of molecular diagnostic procedures for detection of *Phytophthora ramorum* have been reported in the literature. In an effort to evaluate the specificity of 10 of these techniques a standardized DNA library for 317 isolates was assembled that included 60 described species as well as 22 taxonomically unclassified isolates. These were sent blind at a concentration of ca. 10 ng/µl (a concentration greater than would be encountered in field samples, but was used in an effort to fully evaluate specificity) to collaborators to evaluate the various diagnostic procedures. In general the procedures worked well with varying levels of specificity observed among the different techniques. Low levels of nonspecific amplification were observed for the mitochondrial markers and most of the real-time assays based on nuclear markers. The highest level of false positives was obtained with the conventional nested ITS procedure; however, this technique is not standalone and is used in conjunction with two other assays for diagnostic purposes. The results from the APHIS lab and another lab indicated that using three assays improved the accuracy of the results compared to looking at a single assay alone. The SSCP procedure accurately identified *P. ramorum* and was helpful in classification of other isolates to a species level. Given that one of the objectives of the trials was to determine if there would be any false positives, the DNA concentrations that were tested in all but one of the assays (6 to 10 ng/amplification) was higher than would be expected when processing field samples. As a result false positives for some of the assays (in particular real-time assays that had high C_t values) may not be representative of what might be encountered with field samples. Additional evaluations for these samples with a dilution series of target DNA are needed to evaluate specificity at DNA concentrations more reflective of what would be encountered in field assays. Trials evaluating marker performance with samples recovered from the field are in progress.

Key words: Diagnostics, molecular detection.

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Introduction

For effective and accurate diagnosis of *Phytophthora ramorum* it is imperative that that the techniques employed be fully tested for specificity to ensure that false positives will not be encountered when other species are present in infected plant tissue. Likewise, it is important that isolates of the pathogen from a geographic representation of the pathogens distribution also be evaluated to ensure they can be accurately identified. A number of molecular techniques have been developed for detection of *P. ramorum* from infected tissue. However, there has not be a side by side comparison of the techniques with a broad range of *Phytophthora* spp. using the same DNA samples to fully evaluate specificity.

The objective of this research project was to evaluate the specificity of different molecular detection methods for *P. ramorum* using a standardized library of DNA recovered from a range of *Phytophthora* species recovered from geographically diverse areas. Examples of several *Pythium* spp. were included as well. DNA concentration was intentionally higher than would normally be encountered in field samples in an effort to fully evaluate marker specificity and samples were sent blind to all cooperating labs for analysis using marker systems based on spacer regions (the rDNA ITS region, the spacer region between the *cox* I and II gene) or specific genes (beta tubulin, elicitin) and have been configured for use by both conventional and real-time PCR. A technique based on single strand conformational polymorphism of the ITS region was also evaluated for isolate identification.

Materials and Methods

Marker Systems Evaluated

ITS—

Conventional PCR—

- APHIS approved nested amplification – APHIS lab (Beltsville, MD) using procedure of Garbelotto and others (2002)
- Multiplexed amplification using technique of Winton and Hansen (2001) for detection of *P. lateralis*- APHIS lab (Beltsville, MD)

Real-time PCR—

- Technique of the Central Science Lab (CSL) in York, UK (Hughes and others 2006) – K. Hughes lab, CSL
- APHIS approved CSL technique (some procedural differences from the published CSL procedure) – APHIS lab (Beltsville, MD)
- Nested technique of Hayden and others (2006) – Garbelotto lab
- Technique of Bilodeau and others (2007) – Hamelin lab

Single strand conformational polymorphism—

- Technique of Kong and others (2003) modified by running in automated sequencing unit – Kubisiak lab

Nuclear genes—

Real-time PCR—

- Beta tubulin and elicitin genes using technique of Bilodeau and others (2007) - Hamelin lab

Mitochondrial region (cox spacer region)—

- Conventional PCR – using the nested amplification technique of Martin and others (2003) – Martin lab
- Real-time PCR – using the technique of Tooley and others (2006) – Tooley lab

DNA Samples—

DNA was extracted from 317 isolates representing 60 described species and 22 isolates with ambiguous taxonomic classification. Aliquots were coded and sent blind to all participants (a total of 457 samples were sent). The ITS region for all extracts were also amplified and sequenced to confirm the identity of the samples. Some samples were sent multiple times, when this was done they were recoded.

DNA Concentration—

DNAs were provided at a concentration of 10 ng /µl and 1 µl/amplification was used in all procedures with the exception of:

- The nested ITS real-time procedure of Hayden and others (2006) done by the Garbelotto lab - the DNA was diluted 1:100,000 before adding 6.25 µl to the first round amplification (62.5 fg in 25 µl final volume). In the second amplification the first round was diluted 1/500 and 5ul was added to the master mix (final volume of 15ul). Some samples were also run with a dilution of 1/100 (62.5 pg DNA/amplification) for the first round amplification.
- The 3 tests performed by the APHIS lab (APHIS approved ITS nested amplification, the multiplex amplification using the technique of Winton and Hansen (2001), and the APHIS approved CSL real-time ITS technique) - the DNA was diluted 1:10 and 6 µl of this was added to the amplification reaction (6 ng DNA in 25 µl final volume); samples were also run with 1:100 dilutions as well.

Notes on Sample Scoring—

Real-time PCR using the techniques of Bilodeau and others (2007; 3 nuclear loci) and the *cox* spacer region using the technique of Tooley and others (2006) - PCR positives were based on a positive result with a C_t cut off of 40.CSL real-time procedure—PCR positives were scored on a C_t cut off of 36. Below 36 they are scored as positive, between 36 to 40 they are retested (if the tests had been done with plant samples they would be re-extracted when material is available), and samples giving C_t of 40 were scored as PCR negative.

APHIS procedures – conclusions on positive samples were based on the results of the three assays that were performed. A retest of the samples would have been triggered by the sample DNA producing a nested PCR band from at least one of the two tested DNA dilutions (1:10 and 1:100 in double distilled H_2O of the provided DNA stock), not producing a *Phytophthora*-specific Multiplex PCR band with the *P. lateralis* primers of Winton and Hansen (2001), and reacting weakly (C_t-values >30) or not at all with the APHIS approved CSL real-time assay. Similar to the CSL scoring noted above, samples with a C_t of 36 and above must be retested or the results confirmed with the nested ITS procedure. When assaying infected plant material retests that were still ambiguous would have amplicons sequenced to confirm the diagnosis.

Procedure for SSCP—
One microliter of each sample was PCR amplified using the oomycete specific primers ITS-6 and ITS-7 (cited in Kong and others 2003). ITS6 was fluorescently 5´-end labeled with 6-FAM and ITS7 with HEX. A 1:10 dilution of the PCR product was run using the default criteria specified in the "High Throughput Fluorescent SSCP Analysis User Bulletin" from Applied Biosystems on an ABI3100. The separation matrix consisted of 5 percent GeneScan polymer containing 10 percent glycerol and ROX500 was used as the internal migration rate standard. Using this procedure many species had only two main peaks in contrast to the four banded pattern previously reported using slab gel systems (Kong and others 2003). For data analysis, only the largest 6FAM and HEX peaks were scored.

Results and Discussion

The general results that were obtained are outlined in the abstract. To evaluate false negatives a total of 67 samples representing 48 isolates of *P. ramorum* from nine locations were sent to the participants and with few exceptions all were correctly identified. The exceptions were for one or two procedures with specific isolates that were tested a single time; before conclusions about the accuracy of these techniques can be drawn the isolates need to be retested.

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In Vitro Foliage Susceptibility of Canary Islands Laurel Forests: A Model for Better Understanding the Ecology of *Phytophthora ramorum*[1]

Eduardo Moralejo[2] and Enrique Descals[2]

Abstract

The tree species that dominate the cloud-zone forests of Macaronesia, the coastal redwoods of California, the Valdivian forests of Chile, the Atlantic forests of Brazil and the podocarp forests of New Zealand are all examples of paleoendemic species that once had a much wider distribution. They appear to owe their survival to the particular environmental conditions provided by coastal sites or oceanic islands. Some of these coastal areas unfortunately have proved unique scenarios for the establishment and spread of exotic aerial *Phytophthoras* such as *P. ramorum* and *P. kernoviae*. The most reasonable explanation to these invasions is that these ecosystems share climatic and taxonomic affinities with the pathogen's original localities and hosts, but not the defence strategies evolved during the long arms race between the host(s) and pathogen in their original centre. In other words, they have not coevolved into tolerance relationships. Then it would be expected that forest ecosystems showing higher taxonomic affinities to those of the original centre would show more tolerance to the pathogen than those less related. This explanation assumes, however, that (i) there is a common basal defence system in plants (Heath 1991) to which multiple host pathogens, such as *P. ramorum*, are trained to overcome and (ii) a relative ancestral origin of these *Phytophthoras*. We provide the results of *in vitro* foliage inoculations of plant members of the Canary Islands laurel forest as a model for a better understanding of the ecology of *P. ramorum*, as well as a biogeographic link between sudden oak death (SOD) and the possible center of origin of *P. ramorum* in southeast Asia or the Indomalayan archipelago.

In order to differentiate between nonhost resistances due to preformed defence barriers (constitutive defence) and nonhost resistance due to induction of basal defence system (inducible defence), we carried out both wounded and unwounded inoculations. In the first experiment completed in the spring of 2005, leaves were wound inoculated with mycelial plugs. In the second experiments done in the spring of 2006, healthy leaves were inoculated with zoospores. Five isolates, three belonging to the EU1 lineage and two to the NA1 lineage, were used in these assays.

Thirteen tree species belonging to the Lauraceae, Ericaceae, Caprifoliaceae, Aquifoliaceae, Theaceae families showed different responses to *P. ramorum* infection when detached leaves were inoculated with zoospores (unwounded) and mycelium plugs (wounded). They also

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showed diverse capabilities to sustain *P. ramorum* sporulation. Only two species, *Apollonia burbujana* and *Visnea mocanera*, did not form any lesion both in the wound and zoospore inoculations and neither showed hypersensitive response when leaves were examined by dissecting microscope. *Picconia excelsa* and *Heberdenia excelsa* developed necrotic lesions only in the wound treatment; therefore, resistance being due to preformed barriers. All the other species formed necrotic lesions in both experiments. Surprisingly, all species inoculated, even those that did not developed necrosis, sustained to some degree *P. ramorum* sporulation. Interestingly, symptoms on species of the laurel family somewhat reminded those observed on leaves of bay laurel in California. Although most foliage did not show dramatic symptoms when inoculated (tending to tolerance), there seems to be a high risk of establishment if *P. ramorum* is introduced into the laurel forests, considering the plant community composition, structure and prevalent climatic conditions in these ecosystems. Our results suggest that a high host diversity would not decrease the risk of disease transmission.

Based on these and other experiments, two main driving forces are proposed which may determine *P. ramorum* lifestyle: the constraint on short-distance dispersal in a high species diversity forest (e.g. laurel forests), and the fitness costs caused by the need for a multiple-host strategy, which seems to be associated with reduced sporulation (Moralejo and others 2006). Casual observations of asymptomatic infection and sporulation from asymptomatic inoculated foliage are in accordance with the infection and dispersal strategies hypothesized for *P. ramorum*.

Key words: Alien species, nonhost resistance, *Phytophthora* sporulation, disease transmission, fitness cost.

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Monitoring *Phytophthora ramorum* Distribution in Streams Within California Watersheds[1]

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Abstract

One hundred-thirteen sites were established in perennial watercourses and sampled for 1 to 3 years between 2004 and 2006 to monitor for presence of *Phytophthora ramorum* throughout coastal central and northern California watersheds as well as portions of the Sierra Nevada mountain range (Murphy and others 2006). The majority of the monitored watersheds have limited or no *P. ramorum* at this time, but are near the epidemic range of *P. ramorum* and/or are considered high-risk for invasion by *P. ramorum*. Three currently infested watersheds in Sonoma and Mendocino counties were included as a baseline for successful recovery of *P. ramorum*. *Rhododendron* leaves were placed in fiberglass mesh bags, secured to streambanks, and floated on the water surface for 1 to 3 week intervals to bait for *Phytophthora* species (von Broembsen 2002; E. Hansen, personal communication 2003; P. Maloney and J. Davidson, personal communication 2003). The interval time was adjusted year round with the minimum time during periods of warm stream and air temperatures and longer intervals in cold conditions. Recovered symptomatic leaves were described and isolated onto *Phytophthora*-selective medium augmented with 25 mg/L hymexazol to inhibit growth of *Pythium* species (PARP-H). Experiments have shown minimal inhibition of *P. ramorum* and other *Phytophthora* species growth with this concentration of hymexazol (Fichtner and others 2006; Murphy, unpublished data; E. Hansen, personal communication 2004; S. Jeffers, personal communication 2005). Plates were incubated at 18°C for three weeks and checked microscopically twice weekly for growth of *Phytophthora* species; any *Phytophthora*-like organisms were transferred and further examined for identification.

Twenty-seven watersheds were infested with *P. ramorum*, including all sites with a priori knowledge of forest infestation. *Phytophthora ramorum* was found at 14 sites without prior knowledge of forest infestation in Humboldt, Contra Costa, Monterey, and Santa Cruz counties. Forest infestations have thus far been confirmed at only nine of those sites with surveys underway to identify the source(s) of inoculum for the other five sites. Additionally, *P. ramorum* was recovered as far as 7 km downstream from known forest infestations. At two sites in 2006 we observed *P. ramorum* infected plant lesions at high water levels, indicating the possibility of pathogen transmission back onto land from infested water courses. This monitoring has extended the southern range of *P. ramorum* to Willow Creek in Monterey county and the northern range to Elk Creek in central Humboldt county. All sites in the Sierra Nevada remain negative for *P. ramorum* infestation. With culturing and molecular sequencing

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we have also determined the presence of several other *Phytophthora* species throughout these watersheds, including primarily *P. gonapodyides* which was isolated from 60 sites throughout the range of sampling. Sites were monitored year round in 2004 and 2005 and revealed a distinct seasonality associated with *P. ramorum* recovery (fig. 1).

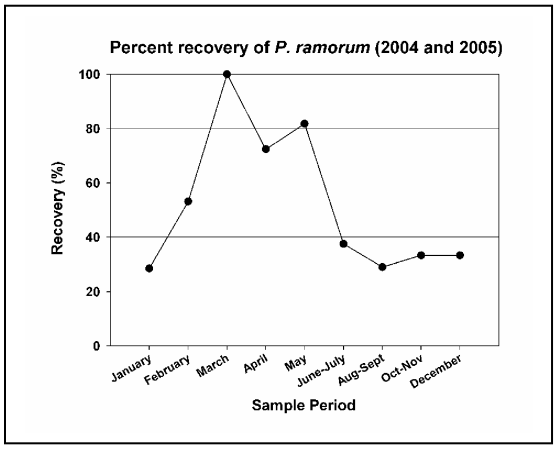

Figure 1—Seasonality of *P. ramorum* recovery from pathogen positive stream sites. Percent recovery for each sample period is averaged from sampling completed in 2004 and 2005.

Stream monitoring provides a useful method of early detection for *P. ramorum* infestation in watersheds. A portion of this work is part of the national *P. ramorum* stream monitoring program supported by the United States Department of Agriculture (USDA)-Forest Service. This project involves many collaborators whose funding, assistance, permission, and guidance make this work possible including: University of California (UC) Davis, UC Cooperative Extension for Humboldt and Del Norte Counties, Cal Poly State University, California Department of Forestry and Fire Protection, UC Angelo Coast Reserve, Landels-Hill Big Creek Reserve, Fairfield Osborn Preserve, UC Berkeley Blodgett Forest Research Station, Hoopa Indian Tribe, Yurok Indian Tribe, East Bay Regional Parks, California State Parks, Sonoma State University, and USDA-Forest Service Forest Health Protection.

In 2007, we will expand monitoring efforts in Mendocino and Humboldt counties to more extensively monitor newly infested and high-risk watersheds. We will additionally survey to locate sources of inoculum and infestation in newly identified positive watersheds. Future studies will include identification of unknown and other *Phytophthora* species occurring in stream courses with molecular analyses. Furthermore, we will work to address research questions related to spread, survival, and quantification of *P. ramorum* in stream courses.

Key words: *Phytophthora ramorum*, water sampling, stream baiting, watershed, California.

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Phytophthora ramorum Early Detection Surveys for Forests in the United States, 2003–2006[1]

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Abstract

Risk-based early detection surveys in U.S. forests were conducted between 2003 and 2006 using 100 m vegetation transects. Thirty-nine states surveyed 3,570 locations in states with endemic *Phytophthora ramorum*; states where the pathogen had been confirmed only in woody ornamental nurseries; and states that had received potentially infected stock but did not confirm the pathogen. A total of 12,699 samples from 44 host and associated host genera were collected and diagnosed for the pathogen using nested or real-time PCR. *Phytophthora ramorum* positive diagnoses were obtained for two samples from San Francisco County, California confirming that the pathogen is not yet widely established outside the regulated areas on the west coast.

Key words: *Phytophthora ramorum*, sudden oak death, survey, risk rating.

Introduction

Diseases caused by *Phytophthora ramorum* in U.S. forest landscapes were first detected in Marin County, CA in the mid-1990s. The endemic range in the U.S. has expanded since then, but in 2007 is still limited to14 central coastal California counties and a small area in Curry County, Oregon (USDA-APHIS. 2007). Despite limited disease distribution in forests, the vulnerability of oak-dominated forest ecosystems in the eastern U.S. is suggested by the demonstration of susceptibility of many closely related native eastern U.S. trees and shrubs in greenhouse inoculation trials (Tooley and others 2007) and via natural infection in Europe (Brasier and others 2004), and by brisk trade in many of these susceptible woody ornamental hosts. This report updates statistics already presented for early detection surveys conducted in 2003 and 2004 (Oak and others 2006).

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Methods

The risk of establishment of the pathogen outside the regulated areas prompted federal and state forest management agencies in seven eastern U.S. states to join in pilot tests of early detection survey methods for forests in 2003. Climatic variables, abundance and distribution of putatively susceptible hosts, and potential pathways of *P. ramorum* introduction on woody ornamental nursery stock were combined in a risk map (Oak and others2006) used to guide sampling intensity. Cooperators were asked to survey 30 locations within their state with an emphasis on high risk areas. Field settings were of two types- wooded nursery perimeter and general forest area. Four-100 m transects were used to survey each location for symptomatic plants. Transect width was not fixed, but rather was determined by host type density. The minimum width was approximately 3 m in extremely dense rhododendron thickets, while the maximum width in sparse oak woodland could be about 30 m. West coast forest host and associated host species do not grow in the pilot survey states, and there was uncertainty as to the full host range of the pathogen. Therefore, target hosts were eastern species in genera of known hosts or associated hosts. Replicates of symptomatic bark, leaf and twig tissues of *Quercus*, *Kalmia*, and *Rhododendron* species were targeted for collection when present, and sent to local and regional laboratories for nested PCR diagnosis of *P. ramorum* according to United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) approved protocol (Levy and others 2004). Replicate testing was conducted as mutual confirmation of suspect positives to minimize the possibility of unnecessary action based on uncertain results.

The survey was implemented operationally in 2004 with mostly minor modifications following the accidental introduction of the pathogen to nurseries and landscapes throughout the country via infected woody ornamentals from a southern California nursery. The most significant modifications were an increase in the number of cooperating states, and expansion of the target host list (11 genera, plus any species displaying suspect aerial *Phytophthora* disease symptoms). Real-time PCR was added as a diagnostic technique after it was certified and approved by USDA-APHIS (DeVries and others2006). Methods remained mostly unchanged in 2005 and 2006.

Results and Discussion

Phytophthora ramorum forest surveys were conducted in at least one year between 2003 and 2006 in a total of 39 states (table 1). Of the 24 states that confirmed *P. ramorum* in ornamental nurseries during this period, only Arizona, Colorado and New Mexico did not conduct surveys, but projected risk in these states is low. Nursery perimeters represented 62 percent of the 3,570 locations reflecting the judgment that these settings were most likely to show symptoms earliest, given the large shipments of potentially infected woody ornamental nursery stock in 2004 and subsequent, smaller, shipments in each year thereafter. Local and regional laboratories diagnosed 12,699 symptomatic tissue samples and only two were found positive for *P. ramorum*. Both of these were *Q. agrifolia* bark samples collected in San Francisco County, California.

Symptomatic tissues from 44 identified genera were submitted for molecular diagnostics. Samples from *Acer* species were the most abundant (table 2) while samples from unidentified genera ranked third in abundance. Symptomatic leaves

were by far the most common tissue type, accounting for 94 percent of all samples. Bark from stem hosts represented less than 5 percent of samples.

Table 1—Summary survey statistics 2003-2006

	Year				
	2003	**2004**	**2005**	**2006**	**Overall**
Cooperating states	7	36	39	36	39
Locations surveyed					
Nursery perimeter	44	881	682	607	2214
General forest	128	304	487	437	1356
Samples Diagnosed	1092	4263	3328	4016	12699
P. ramorum Positive Diagnosis	0	2	0	0	2

Table 2—Frequency and rank of plant genera sampled and submitted for *P. ramorum* diagnosis. Only genera ranked among the top 10 in any survey year are shown

Sample genus	Overall		Year 2003		2004		2005		2006	
	No.	**Rank**	**No.**	**Rank**	**No.**	**Rank**	**No.**	**Rank**	**No.**	**Rank**
Acer	2642	1	11	6	970	1	829	1	832	1
Lonicera	2068	2	17	5	712	3	639	2	700	2
Unidentified	1637	3	284	2	850	2	208	7	295	4
Kalmia	1318	4	363	1	437	4	277	4	241	5
Quercus	1204	5	175	4	295	5	416	3	318	3
Rhododendron	933	6	231	3	257	6	244	5	201	6
Vaccinium	655	7	0		234	7	218	6	203	7
Viburnum	366	8	3	8	108	8	130	8	125	10
Rubus	237	9	0		37	13	4	22	196	8
Prunus	214	10	0		64	10	20	17	130	9
Hamamelis	213	11	0		83	9	71	9	59	16
Aesculus	136	12	0		43	11	46	10	47	17
Castanea	48	20	8	7	27	14	5	21	8	25
Grand Total	**12699**		**1092**		**4263**		**3328**		**4016**	

These surveys resulted in the addition of San Francisco County, California to the regulated area, consolidating the central coastal California distribution of the pathogen to include 14 counties. However, no new detections outside of this area were made. These results further support the conclusion that *P. ramorum* is not widely established in U.S. forests, even in proximity to nurseries where it has been confirmed in infected woody ornamental stock in high risk areas where host type and climate are conducive to disease development. However, not all infected plants were intercepted at the nurseries, and it is certain that some are planted in residential and commercial landscapes in high risk areas. Continued annual introductions from 2003 through 2007 have occurred. The length of latent periods between introduction, establishment in native vegetation, and detection are unknown. These facts dictate continued early detection efforts to maximize the efficacy of eradication of new

outbreaks. Recent advances in sampling of waterways for *P. ramorum* will result in changes in national early detection survey protocols. Transect surveys will be replaced with water sampling using rhododendron leaf bait in 2007.

Acknowledgments

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Implementation of a Thinning and Burning Study in Tanoak-Redwood Stands in Santa Cruz and Mendocino Counties[1]

Kevin L. O'Hara[2] and Kristen M. Waring[3]

Abstract

Three silvicultural treatment study sites are being established to examine the effects of thinning and prescribed burning on infection and spread of *Phytophthora ramorum.* Study sites are located in Mendocino and Santa Cruz counties, California. Stands are even-aged redwood/tanoak mixtures.

Key words: *Phytophthora ramorum*, *Sequoia sempervirens*, *Lithocarpus densiflora*, thinning, prescribed burning.

Introduction

Thinning and burning effects on infection by and spread of *Phytophthora ramorum* ("sudden oak death" or SOD) are largely unstudied. A series of thinning and burning studies are being established in Santa Cruz and Mendocino Counties, California to examine the effects of these practices on SOD. The objective of this work is to examine the effects of thinning and burning on spread of SOD in infested and uninfested mixed tanoak (*Lithocarpus densiflora* (Hook&Arn.) Rehd.) and redwood (*Sequoia sempervirens* (D. Don.)Endl.) stands. The Santa Cruz sites are located on Soquel Demonstration State Forest in infested stands. The Mendocino sites are at Jackson Demonstration State Forest in uninfested stands.

Study Sites

All study sites consist of mixed redwood and tanoak in predominantly even-aged stands, 80 to 100 years old. Two sites are located at Jackson Demonstration State Forest and a single site is located at Soquel Demonstration State Forest.

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.
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Treatments and Methods

Each of the study stands has been divided into four treatment areas of approximately 1 to 3 ha each (fig. 1). Treatments are planned to include thinning, burning, thinning and burning and a control. Treatments are expected to occur during 2007. The protocol for thinning treatments includes a low thin to reduce stocking by approximately 50 percent. Tanoak and redwood will be thinned including reduction of density within sprout clumps. Thinning will attempt to homogenize stand structure across all three study areas. Burning treatments will be low-severity surface fires with possible supplemental slash treatment in thinned areas. Three plots per treatment have been established to document pretreatment vegetation characteristics. Plots are 0.025 ha and systematically located within treatment areas.

Outlook

These treatments – once implemented – will provide opportunities to examine the effects of density reductions and/or burning on SOD in mixed redwood/tanoak forests. The study design may also present opportunities to examine the effects of these treatments when implemented before infection if the Jackson Demonstration State Forest sites become infested. In either case, these study installations present opportunities to overlay other related studies to examine interactions between stand-level management and SOD.

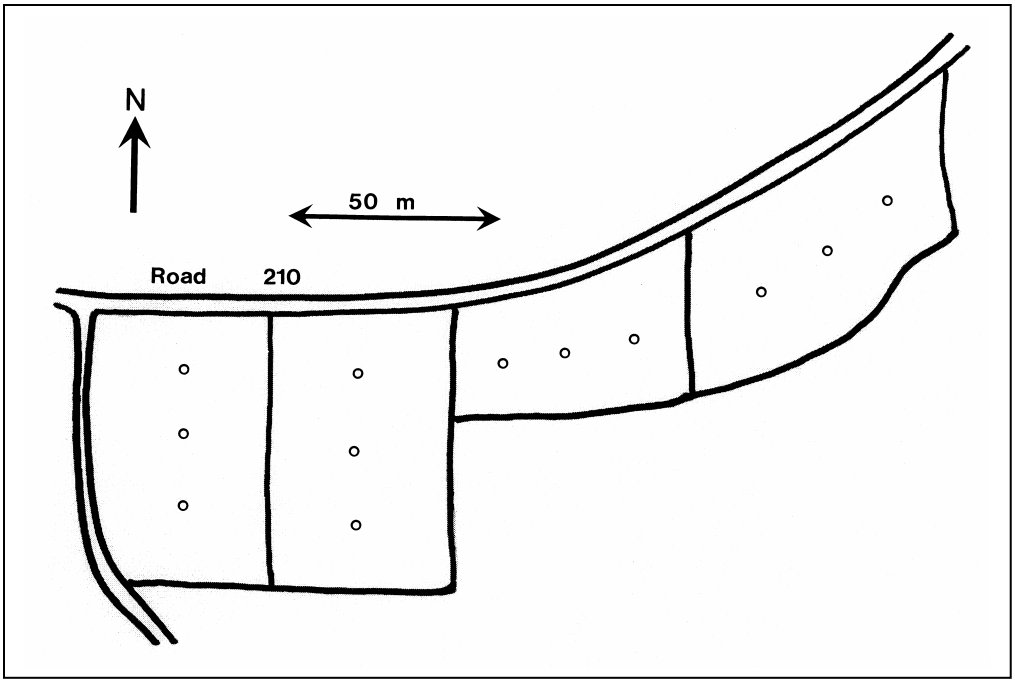

Figure 1—Treatment layout at Jackson Demonstration State Forest showing four treatment areas with approximate locations of sample plots.

Contemporary California Indian Uses for Food of Species Affected by *Phytophthora ramorum*[1]

Beverly R. Ortiz[2]

Abstract

This paper provides a brief survey of contemporary central and northwest California Indian uses for food of regulated hosts and associated species affected by *Phytophthora ramorum*, including recipes from Karuk/Shasta/Abenake elder Josephine Peters. It contextualizes these food uses in terms of their on-going significance in cultural, social and community contexts.

Key words*: Phytophthora ramorum*, sudden oak death, Kashaya Pomo, Bodega Miwok, Dry Creek Pomo, *Umbellularia californica, Quercus kelloggii, Quercus agrifolia, Lithocarpus densiflorus, Corylus cornuta, Vaccinum ovatum, Arbutus menziesii, Arctostaphylos manzanita, Rubus spectabilis.*

Introduction

Since July of 2000, when researchers identified the cause of unusual levels of tanoak dieoffs in Mill Valley, Santa Cruz and Monterey, California, as *Phytophthora ramorum*, or sudden oak death (SOD), California Indians have had to grapple with a new and wide-ranging threat to cultural survival. Not only does this disease kill particular species of acorn-producing trees, but two of those species, black oak (*Quercus kelloggii*) and tanoak (*Lithocarpus densiflorus*), are the most valued acorn-producing species used by California Indians today.

Of the 23 native plant species and one genus that have been designated as regulated hosts for *P. ramorum* as of September 11, 2006[3] and the 18 other associated native plant species that may soon follow as regulated hosts, the author has identified contemporary cultural uses for all but 12. This paper summarizes the use for food among central and northwest California tribal peoples of nine affected species, and presents methods used by elder Josephine Peters to process some of those foods.

The purpose of this paper is to broaden awareness of the continuing importance for contemporary California Indians of the species affected by *P. ramorum*, and to provide a cultural framework through which to consider the impacts and how to respond.

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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[3] The regulated hosts and associated plant species listed in this paper come from the California Oak Mortality Task Force Web site, http://nature.berkeley.edu/comtf.

Those wishing to know more about contemporary uses by California Indians of the species affected by *Phytophthora ramorum*, and how California Indians are responding to its spread, are referred to another paper by the author published in the proceedings of the Sixth California Oaks Symposium: Today's Challenges, Tomorrow's Opportunities, October 9–12, 2006.

Cultural, Social and Community Impacts of *P. ramorum*

> As Indian people we're still using these acorns, and we're still using the tanoak. We've done that since the beginning, when we first walked here, to honor our agreement that we had with those trees to provide us with our food.
>
> -Reno Franklin (Kashaya Pomo), 2007

The cultural, social and community impact of *P. ramorum* for tribal peoples is exemplified by presentations given by Reno Franklin and Eric Wilder of the Kashaya Band of Pomo Indians, who spoke during the Sudden Oak Death Third Science Symposium on March 5, 2007. The Kashaya people have teamed up with researcher Ted Swiecki on a grant to study whether Agrifos® can stem the spread of this disease on the tribe's 16.19 hectare (40 acre) Stewarts Point Rancheria, and other ancestral lands, without affecting the quality and edibility of the acorns. Doctors Rizzo and Garbeletto of the University of California, Davis and Berkeley respectively, have also assisted the tribe in identifying and examining the impact of this disease.

A "culturally practicing tribe" that considers tanoak acorns to be sacred, the Kashaya now refer to one area near the rancheria as "Ground Zero," because *P. ramorum* has killed thousands of trees there. As explained by Reno Franklin about the cultural and social significance of tanoaks for his people, "We still continue to pass on our ceremonies, our traditions, our prayers, and our songs, and some of those songs and prayers and ceremonies are centered around tanoak and these acorns. We...still have roundhouse ceremonies that celebrate and give thanks for what we're taking from those tanoak trees in the form of acorn. You could have a tanoak that's maybe three or four hundred years old where five or six generations of Kashaya families go. Our elders, in this case our grandparents or our parents, our aunties and uncles, will sit down with us and tell us stories underneath these tanoaks. We'll be gathering acorns, and they'll teach us how to sing the songs that are appropriate for gathering, and why we're singing those songs. We'll learn language and stories... It's what we call the University of Kashaya. It's our school. [....] It's a great way for us to connect with each other and all be a part of something. It's a nice picnic day. *P. ramorum* is threatening that. We've got families whose entire gathering areas have been wiped out... And it's hard to take seven generations of a family and remove something like that, and then try and fill that void."

For Kashaya people, the thought of bringing death to tanoaks by gathering plants as their ancestors have for thousands of years is not only "scary," but a "foreign concept." As elaborated by Eric Wilder, "This past season, this was the first time that I really saw the effect of it at our Acorn Festival. We were only able to gather enough acorns for one pot of acorn meal..., so this pathogen has devastated entire areas where our family used to pick. [....] When I was a kid, we'd have all kinds of acorns to

gather. We'd have eight, nine, ten pots of acorn mush out on the table, and then plenty left over that we'd store over the winter until the next season."

Held annually, the Acorn Festival provides a tangible means to give thanks to the trees for providing the acorns. Its four nights of prayer, song and dance culminate in a feast. "What happens," asked Eric Wilder, "when you take that element from your people that is a significant ceremony, and a practice of your people that happened for thousands and thousands of years, and it's suddenly gone? ...The acorn impact is just one element of it. It's in our medicinal plants. It's in our [other] foods. It's in our technical plants.

"In our traditional belief, when we go out and we gather these acorns and anything from the land, the Creator has put that here for us... This is a sacred ceremony that we do... According to the teachings of our people from thousands and thousands of years, if you don't respect the creation, and we don't follow those rules that we were given to gather, this is the kind of thing that will happen... In the traditional people's view...creation's showing us what happens when you don't respect it..., so we feel like we're...responsible for what's happening, too... We're not pointing fingers at other people and saying, 'You brought this pathogen to us.' ...It's here, and it's affecting everything that we use, and so the tribe's position is, 'What can we do? Can you help us? And what can we do to help you?' [W]e all need to work together to try to find a solution to this pathogen. [....] You are part of this land with us. We are expected to follow certain rules, and so, as far as for us, we need to remember who we are and where we come from."

The Stewarts Point Rancheria encompasses an old village site called Huckleberry Heights in the Kashaya language, and the people continue to use huckleberry, now a *P. ramorum* host species, for food. Their word for tanoak translates "beautiful tree." As explained by Reno Franklin, "[W]e cared a lot about this tree to give it such a pretty, descriptive name. We romanticized this tree, and we still continue to." The Kashaya use California bay laurel, a primary host species, both for food and ceremonially. About bay laurel and tanoak, Franklin had this to say, "We're talking about the two things that are so sacred to our tribe, and one [bay laurel] is killing the other [tanoak]... [It's] culturally devastating to have to worry about this disease that's coming in with our medicinal plants and kills other medicinal plants. It's a really scary concept to an Indian person."

Community impacts include the need to cut down dead tanoaks that threaten to fall on homes, and an inability to share wood used in household wood stoves, since one household may have the disease in their backyard, but another down the street may not. As Franklin explained, "That's a foreign concept to not share with our neighbors, especially on the reservation."

The Kashaya are but one of many California tribes who use species affected by *P. ramorum* in their day-to-day lives. Those individuals raised on the resultant foods commonly long for them when they are sick or dying. Earlier this year, for instance, the dying wish of one Dry Creek Pomo woman was to eat "acorn" one last time. The relative who provided that acorn, Kathleen Smith (Dry Creek Pomo and Bodega Miwok), was the second generation in recent memory to fulfill a dying wish for acorn. Her late father Steven Smith was the first (Smith 2007, personal communication).

Such foods, whose processing has been readapted to fit within the context of modern life, remain precious and enduring. A summary of the foods, and three "recipes," are offered below in the hope that they may bring greater awareness to the ongoing use by California Indians of species affected by *P. ramorum*, and suggest another area of consideration when conducting research about this pathogen.

The Foods

Following is a summary of the use for food of both regulated hosts and associated species.

California bay laurel, aka pepperwood (*Umbellularia californica*): In northwest California, roasted nutmeats (peppernuts) eaten for enjoyment, to prevent allergies in the spring, to prevent colds and flu in the fall, and to relieve colitis and ulcers (Ortiz and others 2006: 68-69[4]). In central California roasted nuts pounded, formed into balls and elongate shapes, and eaten for enjoyment (Ortiz 1989: 25, Smith 2004).[5]

Salal (*Gaultheria shallon*). Berries eaten raw or canned (McCovey 2006[6] Ortiz and others 2006: 218).

California black oak (*Quercus kelloggii*), Coast live oak (*Quercus agrifolia*), and Tanoak (*Lithocarpus densiflorus*). Acorns used for food throughout California.[7] In northwest California, the "old timers" relished garnishing their acorn with roasted peppernut halves.[8] For a Yosemite Miwok/Paiute method see Ortiz 1991.

California hazelnut (*Corylus cornuta*). Nuts eaten raw, dried like walnuts, or baked (Colegrove 2006[9], McCovey 2006, Ortiz 1996/97: 27-28, 29, 30, 1998: 24-25, Ortiz and others 2006: 15-16, 30, 68, 140).

Evergreen huckleberry (*Vaccinum ovatum*). Berries eaten raw, canned and cooked in pies and "duff" (sweetened dough balls cooked in sweetened, thickened berries, spiced with cinnamon) (Ortiz 2000:21; Ortiz and others 2006: 63, 64, 112, 148). Berries considered a staple by the Karuk and frozen for year-round use (Glaze 2003[10]).

Madrone (*Arbutus menziesii*). Dried berries pounded, then water added to make a sweet drink (McCovey 2006).

Parry manzanita/Manzanita (*Arctostaphylos manzanita*). To make a sweet cider, powdered berries soaked in water, water dripped through powdered berries, or

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[4] Ortis, Beverly; with Bryan Colegrove; Dwayne and Patricia Ferris; Zona Ferris; Wendy Ferris George; LaVerne Glase; Holly Hensher; Jennifer Kalt; Deborah McConnell; Kathy McCovey, and Kenneth Wilson. 2006. The first full moon in April: Josephine Grant Peters (Karuk, Shasta, Abenake), her life, plant uses, and cultural knowledge; 285 p. Unpublished.
[5] Field data 1983 to 1996.
[6] McCovey, Kathy, Karuk cultural consultant. [Taped interview with Beverly R. Ortiz.] 13 July 2006.
[7] Field data 1988 to 1992.
[8] Field data 2001 to 2005.
[9] Colegrove, Bryan, Hupa/Yurok/Karuk cultural consultant. [Taped interview with Beverly R. Ortiz] 13 July 2006.
[10] Glaze, LaVerne, Karuk cultural consultant. [Taped interview with Beverly R. Ortiz.] 28 June 2003.

powdered berries wrapped in cloth and soaked in water (Ortiz 1996/97: 27, Ortiz and others 2006: 63, 167-168).[11]

Salmonberry (*Rubus spectabilis*). Berries eaten raw (Ortiz and others 2006: 219). Berries canned.

Recipes

The recipes that follow were compiled from field research conducted from 2001 to 2005 with Karuk/Shasta/Abenake elder Josephine Peters. Her words are quoted in italic typeface. For other recipes utilized by contemporary California Indians to process these foods, see Ortiz 1989, 1991, 1996/97, and Smith 2004.

Acorn Recipe

A "good crop" of "acorn" occurs every fourth year, so Josephine gathers more than a year's supply. *We always use tanoak when it's plentiful.* Dry acorns in a box near the stove for a month or so. If gathered before the rains, they'll already be somewhat dry, and can be laid rather thickly in the box. Turn the nuts once or twice daily, whenever you think about it, so that the acorns in the middle don't mold. Crack the nuts with a hammer, butt end up. If you crack the nuts while they're fresh, they turn an unsightly, gray color due to bruising. When dry, the nuts virtually pop out of the shell. Use last year's nuts with a few of the fresh to "boost up the taste." With tanoak the skin comes right off, so there's no need to winnow. With white oak, the skin stays on. *You have to break it off*. Run the nuts through a Miracle Flour Mill, using the smallest gauge, so the flour will be as fine as possible. When fine, the flour will "soak" (leach) in a couple of hours. When the flour has big chunks, it takes longer to soak.

Prop a relatively finely woven twined basket atop a colander in your kitchen sink. Place a cloth atop the basket, and the acorn flour atop this, as much as an inch and a half deep depending on the amount of acorn to be leached. The cloth must be "soft," or water won't leach through it. Josephine uses an old flour sack. A sheet isn't soft enough. Drip tepid water through the flour for about two hours. It removes a yellowish-colored oil, and speeds up the leaching process. If only cold water were used, leaching would take all day. In place of a water break, Josephine changes the position of her faucet from time to time, so the flour will leach evenly. As needed, after two hours, she lets cold faucet water drip through the acorn flour until not a hint of bitterness (tannin) remains. *People who leave the bitterness in turn others against ever eating it. I always take all mine out.* Cook in a stainless steel pot, stirring with a big, wooden spoon lest the acorn be burned on the bottom. Never use aluminum, as acorn will react with the metal. A cup of acorns will make a quart of mush. The cooked "acorn soup" should have a "pretty thick," mush-like consistency. When people prefer it thinner, they can always add water to it.

Peppernut Recipe

While growing up, Josephine and her siblings ate roasted peppernuts to become immune to flu and bad colds. As a child, she learned to fix them herself. Josephine usually gathers peppernuts in the middle of October, right after hunting season, when they fall from the trees. The harvest varies from year to year and tree to tree. *They*

[11] Field data 1980 to 2006.

always say every four years is a good nut crop. After arriving home from the harvest, Josephine squeezes the nuts out of their outer, soft hull, which she considers too bitter to eat. The skin's oily residue helps seal the shell, so the nuts keep longer. *I've had a lot of nuts here for three or four years.* Josephine places the nuts several deep, or about one-and-a-half inches thick, in three special "boxes" she once used for sifting clay when she belonged to a Hoopa-based pottery guild. The boxes consist of a 12 × 12 frame of boards about 6 inches (15.24 cm) high with a screen that runs underneath. Josephine sun dries the nuts during the day, propping the boxes up off the ground with sticks, so there's ample ventilation through the screen. To insure all the nuts dry well, she periodically stirs them around, and brings the boxes in at night. Once dry, after about two weeks, Josephine transfers the nuts into a cardboard box, paper bag or jar, whatever's available for storage. Alternatively, dry the nuts in a basket one nut layer thick for three to four days before roasting. Stored in the shell, the nuts stay "soft," or, put another way, don't get "too old." Josephine begins eating hers about a month after gathering them, nearer the cold and flu season. The nuts will keep for a year.

Roast peppernuts in a 250 degree oven in a pie tin or cookie sheet for 45 minutes to an hour. Open the oven every five to ten minutes or so, and shake the cookie sheet, so the nuts roll. *It takes the bitterness out if you cook it slow. We keep shaking them up so they roast evenly.* Without shaking, the nuts may burn on one side. Josephine determines when the nuts have been properly roasted by the color of the shell, which turns from a yellowish tan to a light brownish-black to a dark brown. Although hot when removed, the nuts cool fairly quickly. If cooked too long, they'll burn, ruining their flavor. Cooked properly they retain a slight hint of their original bitterness, nothing that detracts from the taste. Josephine cracks the thin shells with her teeth. The nut separates into two halves. A papery skin between the halves comes right off. Peppernuts taste similar to a coffee bean. Josephine eats hers plain, or adds them into acorn soup. Eat the roasted nuts within a season. They absorb moisture through the shell, and eventually become soft and chewy, which damages the taste. Josephine stores roasted ones in a jar.

Manzanita Berry Cider Recipe

Gather manzanita berries off fire roads, where they're clean. *We go up in the mountains and pick, if we can beat the bears to them.* [laughs] Pick large clumps of berries by the handful. Remove the stems by rolling the berries in a circular, open-work basket woven close enough to contain the fruit, but open enough for the stems to fall through. Josephine's mother made a sweet drink from manzanita berries by pounding the red, ripened berries in a wooden bowl until the skin powdered. *Of course they've got a lot of seed in them, and not much powder. She'd pound it all up, and put it in a gallon jar. She used to call it cider. It made a nice drink… We'd drink it like we do Kool Aid.* The sweetness leached from the powder as it sat in cold water in the jar. Once the water was sweet, she strained out the powder through a flour sack. Josephine powders her berries by putting them through the biggest chopper in an old meat grinder. She sifts out the hard seeds, some of which get broken up, with a screen sifter or handled sieve. She soaks the powder in a big jar, stirring it up before drinking.

Conclusion

The spread of *P. ramorum* into several coastal and near-coastal, California counties, threatens a vital, thousands-of-years-old relationship between people, cultural heritage, plants and their homelands. Cultural, social, community, emotional, spiritual and historical ties connect people to their homelands, and traditionalist California Indians feel an obligation to Creation, and the Creator, to continue to use plants within these homelands for food and other cultural purposes, while care taking the plants in culturally proscribed ways.

This brief survey of the on-going significance of host and associated species affected by *P. ramorum* for food by California Indians is intended to call attention to some of the lesser-known impacts of this pathogen. As California Indians grapple with the cultural and ecological implications of *P. ramorum*, they face new challenges in the continuance of their cultures. They are currently reaching out to researchers to help them examine and respond to these challenges.

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A High Throughput System for the Detection of *Phytophthora ramorum* in Susceptible Plant Species: A Preliminary Report[1]

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Abstract

Phytophthora ramorum is a pathogen of regulatory concern in North America and Europe. In 2004, potentially infected plants were shipped from large, wholesale nurseries on the West Coast (California, Oregon, and Washington) throughout the U.S. This prompted a nationwide survey effort and the adoption of a federal order requiring mandatory inspection and testing of all West Coast nurseries shipping *P. ramorum* host and associated host plants (HAP) interstate. In Oregon, this required the testing of 51,645 samples from 1,034 growing areas and 79,930 samples from 1,394 growing areas in 2005 and in 2006, respectively. Because all testing must be completed before nurseries can ship HAP interstate, the Oregon Department of Agriculture developed a high throughput system using a 96-well format to enable testing of large numbers of samples in accordance with federally validated protocols (ELISA, nested PCR, and qPCR). To verify the efficacy of the system, healthy leaves from four HAP species were wounded and then artificially inoculated with *P. ramorum*; healthy control leaves were wounded and then inoculated with a sterile agar plug. Samples were collected from the inoculated and control leaves and placed into 10 X 96 collection microtubes. Subsamples from each HAP were bulked five per microtube in varying ratios of inoculated to non-inoculated tissue (5:0, 1:4, and 0:5). Sample tissues were macerated and tested with ELISA according to the manufacturer's protocol. The OD readings of all inoculated samples were consistently 5X the negative control. All non-inoculated controls were below the 2X threshold with one exception. In the second replicate, the OD reading of the *Pieris japonica* non-inoculated control was >2X the negative control. DNA was then extracted from the remaining sample tissue in the GEB2 buffer. All inoculated samples were positive using nested PCR while non-inoculated controls were negative. Sample DNA was then tested with qPCR. All inoculated samples were positive while all non-inoculated controls were negative with one exception. In the first replicate, the *Camellia* non-inoculated control had a Ct value of 40.08 for the *P. ramorum*-specific probe. According to USDA protocol, this sample would be tested with nested PCR to confirm this negative test result. The high throughput, 96-well format system was also used successfully with environmental samples. Samples from four HAP species and six HAP genera were identified as positive with ELISA and subsequently as *P. ramorum*-positive by nested PCR and/or qPCR. USDA officially confirmed these test results. These preliminary results indicate that the high throughput system successfully detected *P. ramorum* in infected plant tissue using the USDA-validated ELISA, nested PCR, and qPCR protocols.

Key words: High throughput testing, ELISA, nested PCR, qPCR.

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Introduction

Phytophthora ramorum Werres, Man in't Veld, & de Cock is a pathogen of regulatory concern in North America and Europe. The disease is established in 14 coastal counties in California and has been detected in Curry County, Oregon (Goheen and others 2002, Rizzo and others 2002). In 2004, *P. ramorum* was detected in large, wholesale nurseries on the West Coast that shipped potentially infected plants throughout the U.S. (Tubajika and others 2006). The shipment of potentially infected plants prompted a nationwide survey effort for this pathogen in nursery stock and prompted the adoption of a Federal Order requiring the mandatory inspection of all California, Oregon, and Washington nursery stock shipped interstate (USDA 2004b). Nurseries growing host and associated plants (HAP) had to be inspected and a mandatory number of samples collected for testing. Nurseries growing non-HAP had to be inspected; samples were collected for testing if suspicious symptoms were found.

Oregon has over 2,100 wholesale nursery and greenhouse operations that generate about $877 million in gross sales (USDA and ODA 2006). Over 80 percent of the nursery stock produced is exported. Many of these nurseries have multiple growing sites throughout the state. Each growing site with HAP present must be inspected and tested for *P. ramorum*. Since the inception of the Federal Order, this has required the testing of tens of thousands of samples from thousands of growing sites (fig. 1). Because all testing must be completed before nurseries can ship HAP interstate, the Oregon Department of Agriculture (ODA) has been developing a high throughput system that enables testing of large numbers of samples in accordance with the federally validated protocols. The system is designed to work with the ELISA and molecular detection protocols for *P. ramorum*.

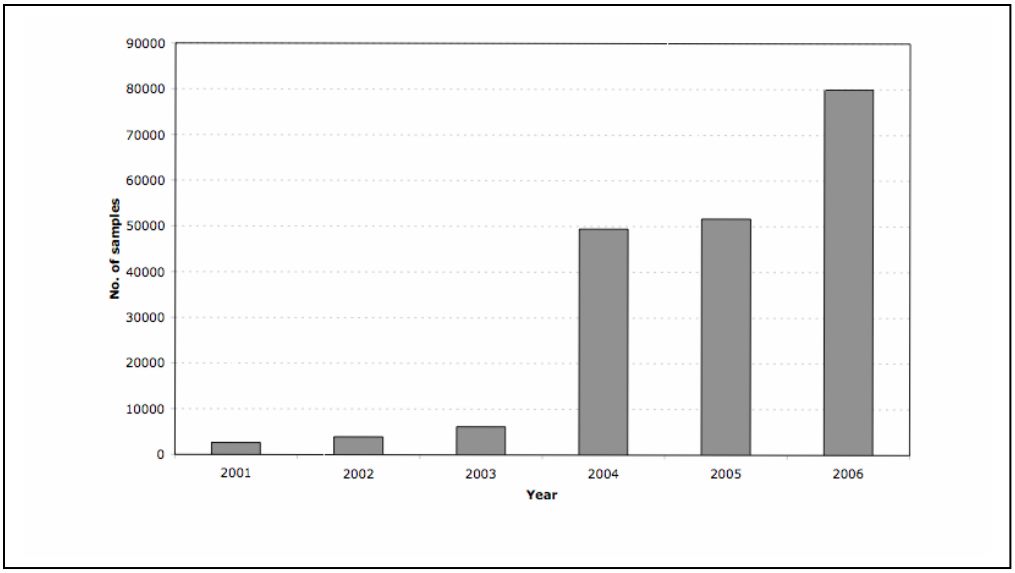

Figure 1—Number of nursery samples tested for *P. ramorum* since 2001.

A sterile hole punch was used to remove a 6 mm diameter disk of infected leaf tissue from each leaf. The leaf disk was taken from the disease margin whenever possible. For all leaves, the area where the mycelial or V8 agar plug was placed on the leaf was avoided.

The leaf disks were placed in a 96-well extraction plate (Qiagen, Inc., Valencia, CA, Cat. No. 19560). For each host, the leaf disks were placed in the microtubes (test wells) in the following combinations of inoculated to non-inoculated tissue: 5:0, 1:4, and 0:5. Two to five replicates of each treatment combination were tested. Eight hundred microliters of sodium azide-free GEB2 buffer (Agdia, Inc., Elkhart, IN, Cat. No. SRA 92600) was added to each test well along with a single tungsten carbide bead. The test wells were sealed to ensure sample integrity and then agitated for 1.5-minutes at 30 MHz in a Mixer Mill. The extraction plates were rotated and the agitation repeated. The extraction plates were centrifuged for approximately 10 seconds at 6000 rpm. The resulting plant extract was then subjected to ELISA testing.

The USDA-validated ELISA testing was performed according to the manufacturer's directions (AgDia Inc., *Phytophthora* instructions for the *Phytophthora* reagent set, m39.4). Three controls were included with each plate: *P. ramorum* from mycelium (positive), the manufacturer's *Phytophthora*-positive, and GEB2 buffer (negative). After 1 hour incubation in the dark, the optical density of the alkaline phosphatase label was read at 405 nm. Per the manufacturer's directions, the positive/negative threshold was set at 2x the optical density (OD) of the negative (buffer) control. Plant extracts were frozen at –20 °C before proceeding to DNA testing.

Total DNA was extracted from the sample extract using the DNeasy Plant Mini and DNeasy 96 Plant kits (Qiagen, Inc., Valencia, CA, Cat. Nos. 69106 and 69181, respectively). The DNA was then subjected to testing with the USDA-validated nested PCR protocol (USDA 2004a) and the real-time PCR (qPCR) protocol developed by the United Kingdom (Tomlinson and others 2005).

The experiments were replicated twice.

Environmental samples were collected from nurseries according to the requirements of the Federal Order and/or the USDA National Survey Protocol for *P. ramorum*. Samples were collected and bulked using the following criteria: HAP genus or species, cultivar, and location (for example, block) within the nursery. At least 40 samples were tested per nursery; five samples were bulked per test well. Samples were processed as described above with the following exceptions: 1) The volume of GEB2 buffer used for the ELISA pre-screen was lowered to 600 µL per test well; and 2) The USDA-validated qPCR protocol was used (USDA 2005).

Results

The OD readings of all samples containing inoculated plant tissues were consistently five times the negative control (table 1). All non-inoculated controls were less than two times the negative control with one exception. In the second experiment, the OD reading for the *P. japonica* non-inoculated control was 0.093 greater than the negative control.

All inoculated samples were positive for *P. ramorum* using the USDA-validated nested PCR protocol and all non-inoculated controls were negative (table 1). Results were similar upon testing with the qPCR protocol with one exception (table 1). In the first experiment, the *Camellia* non-inoculated control had a Ct value of 40.08 with the *P. ramorum*-specific probe.

Table 1—ELISA, nested PCR, and qPCR test results for plant species inoculated with *P. ramorum*

Sample	Ratio[a]	No. of replicates per experiment	Mass (g) (SE)[b]	OD (405 nm) (SE)	Nested PCR	qPCR (Ct)	
						Pram (SE)	COX (SE)
Pieris japonica	5:0	2	0.022 (±0.001)	1.562 (±0.290)	Positive	28.7 (±1.7)	38.2 (±3.6)
P. japonica	1:4	5	0.027 (±0.003)	0.739 (±0.188)	Positive	31.7 (±2.5)	30.8 (±1.7)
P. japonica	0:5	2	0.034 (±0.004)	0.216 (±0.077)	Negative	NA[c]	27.0 (±1.5)
Kalmia sp.	5:0	2	0.037 (±0.000)	2.178 (±0.027)	Positive	31.5 (±5.9)	NA
Kalmia sp.	1:4	5	0.037 (±0.001)	1.487 (±0.406)	Positive	29.2 (±2.2)	29.6 (±1.1)
Kalmia sp.	0:5	2	0.048 (±0.001)	0.155 (±0.023)	Negative	NA	30.4 (±0.3)
Camellia sp.	5:0	2	0.056 (±0.002)	2.163 (±0.348)	Positive	30.4 (±1.6)	37.6[d]
Camellia sp.	1:4	5	0.049 (±0.004)	1.213 (±0.586)	Positive	31.4 (±2.2)	31.0 (±0.9)
Camellia sp.	0:5	2	0.061 (±0.002)	0.103 (±0.004)	Negative	40.1[d]	31.7 (±3.1)
Syringa vulgaris	5:0	2	0.043 (+0.000)	2.542 (±0.097)	Positive	24.2 (±0.3)	NA
S. vulgaris	1:4	5	0.034 (±0.003)	1.804 (±0.483)	Positive	24.8 (±2.8)	33.9 (±2.6)
S. vulgaris	0:5	2	0.043 (±0.001)	0.114 (±0.003)	Negative	NA	25.4 (±2.5)
P. ramorum	Mycelial plug	2	0.096 (±0.000)	1.586 (±0.014)	Positive	28.34 (±1.1)	NA
AgDia positive	—[e]	2	—	1.226 (±0.006)	—	—	—
Negative (buffer)	—	2	—	0.089 (±0.001)	Negative	NA	NA

[a] Ratio of inoculated to non-inoculated plant tissue.
[b] Standard error.
[c] NA means no amplification occurred.
[d] Ct from one replicate. There was no amplification in the second replicate.
[e] Not tested.

Environmental samples were also tested with the high throughput system (partial dataset shown in table 2). The high throughput system was used with ELISA to successfully screen multiple plant species for *Phytophthora* infection. Subsequent DNA testing with the USDA-validated nested PCR and/or qPCR protocols successfully detected *P. ramorum* in the following HAP: *Abies concolor* (Gord. & Glend.) Lincl. ex Hildebr., *Acer macrophyllum* Pursh, *Camellia* sp., *Gaultheria shallon* Pursh, *Kalmia* sp., *Magnolia* sp., *Pieris* sp., *Rhododendron* sp., *S. vulgaris*, and *Viburnum* sp.. *P. ramorum* was also detected from soil and potting media bait leaves using the high throughput system. These results were officially confirmed by the USDA-APHIS at their testing laboratory in Beltsville, MD (data not shown).

Table 2—*P. ramorum* testing results for samples collected from a subset of Oregon nurseries

Nursery	Host	No. of samples	OD (405 nm) (SE)[a]	Nested PCR (ODA)	USDA Confirmation
---034	*Camellia* sp.	10	0.102 (±0.002)		
---034	*Pieris japonica*	15	1.890 (±0.265)	Positive	Negative
---034	*P. japonica*	15	0.394 (±0.226)	Negative	
---610	*Camellia* sp.	15	0.102 (±0.002)		
---610	*P. japonica*	10	0.106 (±0.004)		
---610	*Viburnum davidii*	15	0.422 (±0.264)	Negative	
---065	*Abies grandis*	20	0.107 (±0.004)		
---065	*Pseudotsuga menziesii*	20	0.119 (±0.006)		
---415	*Pseudotsuga menziesii*	10	0.425 (±0.217)	Negative	
---415	*P. menziesii*	10	0.136 (±0.034)		
---415	*P. menziesii*	10	0.152 (±0.006)		
---415	*P. menziesii*	10	0.194 (±0.033)		
---734	*Arbutus menziesii*	10	0.112 (±0.000)		
---734	*Rhododendron*	10	0.107 (±0.004)		
---734	*Taxus brevifolia*	10	0.104 (±0.001)		
---734	*Vaccinium ovatum*	10	0.115 (±0.005)		
---327	*Kalmia latifolia*	10	0.873 (±0.118)	Negative	
---327	*Pieris japonica*	10	0.786 (±0.014)	Positive	Negative
---327	*Rhododendron*	10	0.133 (±0.010)		
---327	*Rhododendron*	10	0.745 (±0.048)	Positive	Positive
---327	*Rhododendron*	10	0.159 (±0.032)		
---327	*Viburnum tinus*	10	0.110 (±0.004)		
	Positive control		1.041 (±0.018)	Positive[b]	
	Negative control		0.100 (±0.001)	Negative[c]	

[a] Standard error.
[b] A *P. ramorum* positive control was used for nested PCR.
[c] A no template control was used for nested PCR.

Conclusions

The high throughput system was used successfully to detect *P. ramorum* in four known host species that were artificially inoculated with the pathogen. The system worked well with all three USDA-validated protocols (ELISA, nested PCR, and qPCR). Because of the large number of samples that needed to be tested (Fig. 2), we investigated the possibility of bulking samples. In an initial, internal study (data not shown), we tried bulking 10 samples per test well and were able to successfully detect *P. ramorum* infections. However, ELISA is known to be less sensitive than PCR for detecting plant pathogens (for example, Lee and others 2001) and we became concerned about the potential for false negatives in samples with low levels of infection. Because of this concern, we reduced the number of samples bulked per test well to five in this study. This allowed for consistent detection of a low infection level (one of five samples infected) with the ELISA pre-screen. When environmental samples were tested with the high throughput system, *Phytophthora* was successfully detected in multiple HAP species including conifers at 15 percent of the nursery sites surveyed in both 2005 and 2006 (Oregon Department of Agriculture, 2005 Plant Division Annual Report and 2006 Plant Division Annual Report available at http://oregon.gov/ODA/PLANT). Subsequent testing with nested PCR and qPCR successfully detected *P. ramorum* in four plant species including the conifer *Abies concolor* and in six plant genera. All PCR results were officially confirmed by USDA, indicating *P. ramorum* could be detected in bulked environmental samples. To our knowledge, ODA is the only regulatory laboratory at this time that has the equipment necessary for this type of high throughput system. Replication of this work in one or more other laboratories will be needed to validate the system.

Figure 2—Inoculated *Kalmia* leaves.

Because samples were bulked in a 96-well format for testing, our laboratory was able to process tens of thousands of samples in a timely and accurate manner. In terms of manual labor costs for sample processing, this translated into a savings of about $150,000 over a 2 year period. The 96-well format could also result in long-term savings by automating the testing procedures. Automation could also decrease the risks of cross-contamination or environmental contamination of samples, potentially reducing the chance of false positives.

The USDA-validated ELISA test requires a 1:10 (w:v) sample to extraction buffer ratio for protein extraction. In our artificial inoculation study, the average mass of the plant species tested was 0.041 mg, which would require an extraction buffer volume of 410 μL. Because there are more than 100 plant species susceptible to natural infection by *P. ramorum*, we adjusted the volume to 600 μL of the extraction buffer GEB2 per sample tested to account for any species differences in mass. This volume was sufficient to meet the 1:10 (w:v) requirement for all HAP species tested and also worked well with the DNeasy 96 Plant Kit for DNA extraction. Based on the Ct values for COX in the qPCR test and the results from the nested PCR test, the DNA extracted from the ELISA sample extract was of sufficient quality for PCR amplification. This showed that DNA was successfully extracted from sample tissue that was initially macerated in GEB2 buffer. Thus, sample integrity was maintained as the exact same sample tissue was tested with all three USDA-validated protocols. This is important because *Phytophthora* species, like other plant pathogens, may grow unevenly along the disease margin. By testing the exact same ELISA-positive tissue, diagnosticians can be assured that a *Phytophthora* is present in the sample for DNA extraction and testing.

In a study performed by USDA on a cultivar of *C. japonica* L., researchers determined that a positive/negative threshold of 2X a negative (healthy plant) control was necessary to reduce the rate of false-negatives detected with ELISA (Bulluck and others 2006). In this study, we chose to use the extraction buffer as the negative control for ELISA testing. Using the manufacturer's recommended positive/negative threshold of 2X the negative (buffer) control's OD reading resulted in a false ELISA positive from healthy *P. japonica* plant tissue in the controlled inoculation study (Table 1). If a healthy plant control produced such a high OD reading during the testing of environmental samples, it could result in false ELISA negatives for infected environmental samples because the OD reading of the infected environmental sample would be less than 2X the healthy plant control. In our study, The OD readings for infected plant tissue were consistently 5X the negative control for all artificially inoculated HAP species tested and consistently ≥3X the negative (buffer) control for environmental samples tested. In a previous study that examined the sensitivity of *Phytophthora*-specific immunoassay kits, a positive/negative threshold of 0.3 was used with limited success (Pscheidt and others 1991). Our data suggest that there is a need for a comprehensive ELISA analysis of additional HAP species to determine an appropriate positive/negative threshold that eliminates false positives from healthy plant tissue while minimizing the rate of false-negatives. Such a study could be used to identify an appropriate healthy plant tissue control for the testing of multiple susceptible species for *Phytophthora*.

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Global Gene Expression Profiles of *Phytophthora ramorum* Strain Pr102 in Response to Plant Host and Tissue Differentiation[1]

Caroline M. Press[2] and Niklaus J. Grunwald[2]

Abstract

The release of the draft genome sequence of *P. ramorum* strain Pr102, enabled the construction of an oligonucleotide microarray of the entire genome of Pr102. The array contains 344,680 features (oligos) that represent the transcriptome of Pr102. *P. ramorum* RNA was extracted from mycelium and sporangia and used to compare gene expression across tissue types and in the presence of the host (*Rhododendron* sp.). The purpose of the experiment was to identify genes whose expression was responsive to tissue types and upon exposure to the host plant for further study. Gene expression studies were performed using a Nimblegen microarray. Genes were determined to be differentially expressed between tissue types if they were statistically significant P=0.05 after false discovery rate correction and resulted in a greater than 20-fold change in gene expression selecting only those genes with the greatest response to tissue changes or host influence. In the comparison between mycelia and sporangial tissues, 263 genes demonstrated a greater than 20-fold change in gene expression. Of those genes, 52 genes were significantly downregulated in sporangia as compared to mycelium and 214 genes were significantly upregulated in sporangia as compared to mycelium. Several of the differentially expressed genes appear to be of the same type as those in a similar study in *Phytophthora infestans* by Kim and Judelson (Eukaryotic Cell, 2003, 2:1376–1385) and include genes involved in cell wall restructuring, cell division and signaling functions. Not surprisingly, several genes involved in energy production, electron transport chains and growth are reduced in sporangia. Fifty percent of the genes with differential expression have not yet been classified as to function in the current annotation.

Key words: *Phytophthora ramorum*, gene expression analysis, microarray, sudden oak death.

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Susceptibility of Some Native Plant Species From Hawaii to *Phytophthora ramorum* [1]

Paul Reeser,[2] Everett Hansen,[2] Wendy Sutton,[2] Jennifer Davidson,[3] and Jennifer Parke[2]

Abstract

The remaining native flora of Hawaii are under continuing pressure from habitat loss and exotic, invasive organisms, including animals, plants, and pathogens. In order to assess the risk to *P. ramorum*, we inoculated seedlings of *Metrosideros polymorpha* (ohia), *Vaccinium calycinum* (ohelo), *Acacia koa* (koa), and *Leptecophylla tameiameiae* (pukiawe) with the pathogen. Two isolates were used, one from an Oregon horticultural nursery, and one from tanoak in the Oregon infested forest area. A zoospore suspension was sprayed on the intact plants. Plants were inspected periodically and symptomatic plants or plant parts were harvested and photographed. For isolation of *P. ramorum* symptomatic tissue was surface disinfested and plated in semi-selective agar. There was some symptom development in some inoculated plants for all the species tested. However, infection was confirmed by re-isolation of *P. ramorum* only in ohelo, pukiawe, and koa. Detached leaf inoculation also demonstrated susceptibility of *Vaccinium reticulatum*, another species of ohelo.

Key words: Sudden oak death, *Metrosideros polymorpha* (ohia), *Vaccinium calycinum* (ohelo), *Vaccinium reticulatum, Acacia koa* (koa), and *Leptecophylla tameiameiae* (pukiawe).

Materials and Methods

Metrosideros polymorpha (ohia), *Vaccinium calycinum* (ohelo), *Acacia koa* (koa), and *Leptecophylla tameiameiae* (pukiawe) were inoculated with zoospore suspensions of *Phytophthora. ramorum* forest isolate 2027.1 (1.6×10^5 per ml) and nursery isolate 03-74-1 (9.5×10^4 per ml). Inoculum was applied to plants with an airbrush sprayer. Two plants of each species were sprayed with de-ionized water, 15 ml over two plants, to serve as non-inoculated controls. Five plants of each species were sprayed with about 20 ml of the zoospore suspension of either isolate 2027.1 or 03-74-1. Inoculated plants were enclosed in thin white plastic bags for three days to maintain high humidity. The plants were incubated at around 20°C, with full fluorescent light banks at 12 hour photoperiod, and inspected periodically for symptoms. Symptomatic plants or plant parts were harvested and photographed. For isolation of *P. ramorum* symptomatic tissue was surface disinfested with 10 percent household bleach, rinsed three times in de-ionized water, and plated in corn meal

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agar amended with 10 ppm natamycin, 200 ppm Na-ampicillin, and 10 ppm rifampicin (CARP).

Vaccinium reticulatum (ohelo) was tested in detached leaf assays as part of a larger experiment on the susceptibility of *Vaccinium* species and blueberry cultivars to *P. ramorum* (Parke, unpublished). Leaves were collected from greenhouse-grown *V. reticulatum* plants (accession 780.002) at the National Clonal Germplasm Repository, Corvallis, Oregon. Five leaves were dipped in each of three treatments: sterile water (non-inoculated controls), or zoospores from each of two sources of inoculum (nursery isolates and forest isolates) at 10^4 ml-1. The nursery isolates (A1 mating type) were 03-74-D12A and 03-74-1. The forest isolates (A2 mating type) were 2027.1 and 4169. Leaves were incubated flat in moist chambers for 7 days at 19-21 C. *Rhododendron* "Cunningham's White" and *Vaccinium ovatum* were included as positive controls, and *V. macrocarpon* 'Bugle' was included as a negative control. The experiment was conducted twice. At six days, leaves were scanned, and digital images subjected to image analysis (Assess, APS Press, St. Paul, MN) to determine total leaf area and percent necrotic area.

Results and Discussion

There was some symptom development in some inoculated plants for all the species tested. Symptoms were observed six days after inoculation in ohelo plants 1 and 2, and in koa plants 1 and 2. Symptoms in additional plants were observed four weeks after inoculation. However, infection was confirmed by re-isolation of *P. ramorum* only in ohelo, pukiawe, and koa.

In both detached leaf trials, with both sources of inoculum, *V. reticulatum* leaves were 100 percent necrotic. Non-inoculated controls and inoculated *V. macrocarpon* 'Bugle' leaves were not necrotic. Mean percent necrotic area was 41 percent and 82 percent for inoculated *Rhododendron* 'Cunningham's White' and *V. ovatum*, respectively.

Acknowledgments

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Phytophthora siskiyouensis, a New Species From Soil and Water in Southwest Oregon[1]

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Abstract

An unknown *Phytophthora* species was recovered from rhododendron and tanoak leaf baits used for monitoring streams and soils in Southwestern Oregon for the presence of *Phytophthora ramorum*. Isolates of this species yielded ITS-DNA sequences that differed substantially from other *Phytophthora* sequences in GenBank. Morphological features also differed from descriptions of known *Phytophthora* species. Based on the combination of unique morphology and unique ITS sequences, a new species is proposed. The new species, *Phytophthora siskiyouensis*, is homothallic, with globose to sub-globose oogonia, which may be terminal, sessile or lateral-intercalary. Antheridia are capitate and mostly paragynous, but sometimes amphigynous. Oospores are mostly aplerotic. Sporangia are ovoid to reniform, with apical, sub-apical, or lateral semi-papillae (occasionally more than one). Sporangia are terminal, sub-terminal, or occasionally intercalary on unbranched sporangiophores, with basal, sub-basal or lateral attachment. Sporangia are weakly deciduous, with variable length pedicels. This combination of characters clearly separates this taxon from other known *Phytophthora* species. *Phytophthora siskiyouensis* refers to the geographic region of origin.

Introduction

Phytophthora species are well known as pathogens of agricultural crops and of some forest species. Little is known of *Phytophthora* in natural settings. Ongoing surveys of *Phytophthora* species present in streams and soils in the sudden oak death epidemic regions of Oregon and California have revealed several undescribed *Phytophthora* species. In this paper we describe *Phytophthora siskiyouensis*, which is found in soil and water in the forested areas of coastal southwest Oregon.

Materials and methods

Isolates of *Phytophthora* were recovered from leaf or pear bait pieces plated in corn meal agar amended with 10 ppm natamycin (Delvocid ®), 200 ppm Na-ampicillin, 10 ppm rifampicin, 25 ppm Benlate, and 25 ppm Hymexazol (CARPBH). Isolates were grown on corn meal agar amended with 20 ppm ß-sitosterol (CMAß) to obtain mycelium for DNA extraction and storage as agar plugs in sterile de-ionized water.

Unknown isolates were analyzed by single strand conformational polymorphism (SSCP) of nuclear ribosomal internal transcribed spacer (ITS) and mitochondrial

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cytochrome oxidase (COX) spacer DNA fragments. ITS sequence analysis of isolates from one unique group indicated an undescribed species. Preliminary observations suggested the presence of shared morphological features consistent with a single new species.

For morphological study isolates were grown on clarified V8 agar amended with 20 ppm ß-sitosterol (V8S). Oogonia and oospores were observed on 20 to 30 day-old cultures fixed and preserved in 3.7% formaldehyde. Sporangia were produced by transferring discs from margins of mycelium grown on V8S into natural stream water. Sporangia were fixed and preserved in 3.7% formalin, 10% acetic acid, 0.03% gelatin in 0.05M Na-K buffer (FA-PBG). Specimens were mounted in the fixative and viewed with brightfield illumination on a Zeiss standard microscope with a 63X objective, and measured with an eyepiece micrometer.

Results and discussion

Oogonia (average 25–30 µm) were formed abundantly on V8S in single culture and were usually globose to sub-globose, occasionally much elongated or with funnel shape tapering toward the stalk. Oogonia were terminal on long or short stalks (often bent or kinked), and were frequently sessile and occasionally unilaterally intercalary. Antheridia were predominately paragynous and capitate, with around 10 percent amphigynous. Antheridia were typically terminal, occasionally intercalary, and usually diclinous, attached anywhere on the oogonium. Oospores (average 23–26 µm) were globose to sub-globose, and usually aplerotic, sometimes markedly so in elongated oogonia. Hyphal swellings or chlamydospores were not observed.

Sporangia (average 46–70 µm length by 30–50 µm width) were formed sparsely in V8S agar and abundantly on agar culture pieces placed in stream water. Sporangia varied widely in shape, but were typically ovoid, reniform, or some misshapen variant of these. Overall length to breadth ratio was 1.5, ranging from 1.2 to 2.0 for individual isolates. Sporangia were scarcely semi-papillate with prominent thickening. Semi-papillae were applied apically, sub-apically or laterally, occasionally with two, and more rarely with three, papillae. Sporangiophores were simple, unbranched, short or long, attached basally, sub-basally or laterally to the sporangium. There was often a small hyphal swelling associated with the sporangiophore. Sporangia were typically terminal, but were often sub-terminal and occasionally intercalary, and weakly deciduous with variable pedicel length (average 28 µ, individual pedicels 0 µ to 133 µ).

P.siskiyouensis sporangia resemble those depicted for *Phytophthora quercina*, (Jung et al. 1999) except that they are slightly larger, semi-papillate, and weakly decidous, and are formed singly on simple, unbranched sporiangiophores. Sexual structures also resemble *P. quercina*, except that antheridia may be paragynous or amphigynous and paragynous antheridia are attached anywhere on the oogonium. The oogonial stalk and arrangement of paragynous antheridia are similar to those described for *P. hedraiandra* (de Cock and Levesque 2004).

Colonies on V8S were submerged with a faint radiate pattern and little or no aerial mycelium. Optimal temperature for growth of the nine isolates tested was 25 C. Radial growth rate averaged 7.5 mm/day at 25 C (individual isolates ranged from 6.2

to 8.5 mm/day at 25C). Incubation at 35 C was lethal, and cultures did not recover when removed to 20 C. Isolates which did not grow at 5 C or 30 C recovered when removed to 20 C.

DNA extracts from six *P. siskiyouensis* isolates were amplified with primers DC6 and ITS4. Amplified products were sequenced with DC6, ITS2, ITS3 and ITS4 as sequencing primers. The six *P. siskiyouensis* isolates were identical. Published sequences of other *Phytophthora* species were downloaded from GenBank and aligned using Clustal X v1.81. *P. siskiyouensis* clustered on a branch with *P. capsici* and *P. tropicalis*.

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Phytophthora ramorum in Scotland: Is It All Over?[1]

Alexandra Schlenzig[2]

Abstract

Phytophthora ramorum was found for the first time in Scotland in April 2002 on some *Viburnum tinus* plants in a nursery. Seventeen more outbreaks were confirmed in the same year, all on plants moving in horticultural trade. Phytosanitary emergency measures to eradicate the disease were taken, such as destruction of infected plants followed by movement restrictions for potential hosts in the affected premises and increased monitoring. These measures proved to be successful. Already in the following year the number of outbreaks declined dramatically to six, followed by five in 2004,three in 2005, and none in 2006. A new outbreak in January 2007 brought the number of outbreak sites up to 22. With the exception of one private garden, the disease occurred only in nurseries and garden centres.

Key words*: Phytophthora ramorum,* Scotland, monitoring.

Introduction

Around the year 2000 it emerged that the so called "sudden oak death" pathogen was already present in German and Dutch nursery stock on *Rhododendron* and *Viburnum* since 1993. Concerned about the devastating impact this disease has on oak and tanoak trees in the western United States the Scottish Executive Environment and Rural Affairs Department (SEERAD) in conjunction with the Scottish Agricultural Science Agency (SASA) initiated a survey of nurseries and garden centres in July 2001. The disease was then found in April 2002 on some *Viburnum tinus* plants in a nursery in the East of Scotland. Seventeen more outbreaks occurred the same year.

In May 2002 Scotland followed England and Wales and introduced emergency legislation to prevent the further spread of the disease: the "Plant Health *Phytophthora ramorum* Order". The Scottish emergency measures were replaced by the very similar EU Commission Decision 2002/757/EC from the 1st of November 2003. In brief, the import of susceptible plants and plant material from infested areas of the U.S. is prohibited. Within Europe the movement of listed host plants is controlled and requires a valid plant passport. This EU legislation has been reviewed regularly and amended, for example to include new host plants.

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Surveillance

Survey Sites

Scottish nurseries registered with SEERAD for trading with plants listed in the Plant Health (Scotland) Order 2005 are inspected routinely twice per year. Because of the high number of findings during 2002 the number was increased to four inspections per year. If a nursery bordered on woodland or a park with host plants present, the surrounding area was also observed.

Public gardens and parks were surveyed. To narrow down the search they were chosen on the base of new plantings of *Rhododendron* or *Viburnum* within the last five years or vicinity to outbreak nurseries. Climatic conditions were also taken into consideration. In 2003 and 2004 50 and 80 sites, respectively, were inspected. In 2006 40 landscape sites were targeted.

A Forest survey, concentrating on sites with wild rhododendron undergrowth, was undertaken by the Forestry Commission in early 2004 and included 500 Scottish sites.

Survey Results

Samples taken by the SEERAD inspectors were submitted to SASA. Diagnosis was based on morphological features after plating on V8-PARPNH medium (Jung and others 1996) and conventional PCR as described in Schlenzig 2006.

In 2002, 121 samples were tested for *P. ramorum* and 32 of them were positive, coming from 18 different outbreak sites (fig.1). One of the outbreaks was on a recently planted *Viburnum x bodnantense* in a private garden. All other outbreaks were in nurseries or garden centres. In 2003, 202 samples were taken and 15 of

Figure 1—Outbreak sites in Scotland.

themwere found to be positive, coming from six different outbreaks. Four of them were on sites that had already outbreaks the year before. In 2004 only five out of 206 samples tested positive. These samples came from five different outbreaks, of which only one was a new outbreak site. In 2005, 78 samples were tested of which eight were positive coming from three different outbreaks. All of the sites had already outbreaks in at least one of the previous years. In 2006, 48 samples were taken and none were positive.

Apart from the above mentioned exception in 2002, all outbreak sites were nurseries, garden centres or wholesalers. The pathogen has so far never been found in an area surrounding an outbreak site nor was it found in any of the garden, woodland, or landscape sites. Most commonly found was *P. ramorum* on *Viburnum* with 31 positive samples, especially *V. tinus*; followed by *Rhododendron* spp. with 27 positive samples (table 1). The only other host species found was *Syringa vulgaris*, which was confirmed as new host (Beales and others. 2004).

Table 1—Host plants for *Phytophthora ramorum* in Scotland

Host	*Number of findings*
Viburnum tinus	23
Viburnum x bodnantense	3
Viburnum farreri	3
Viburnum davidii	1
Viburnum plicatum	1
Rhododendron spp.	27
Syringa vulgaris	2

Discussion

The introduced measures against *P. ramorum* have been very effective in Scotland. Although originally the pathogen was detected in a relative high number of nurseries, the number of outbreaks decreased already remarkably in the next year and even no outbreaks at all in 2006 (Fig. 2). Essential for the success was the detection of the disease in an early stage before it was able to establish itself. Scotland was one of the first countries in Europe to find *P. ramorum*. Supported were the emergency measures through the peripheral location of Scotland with limited trade of host plants.

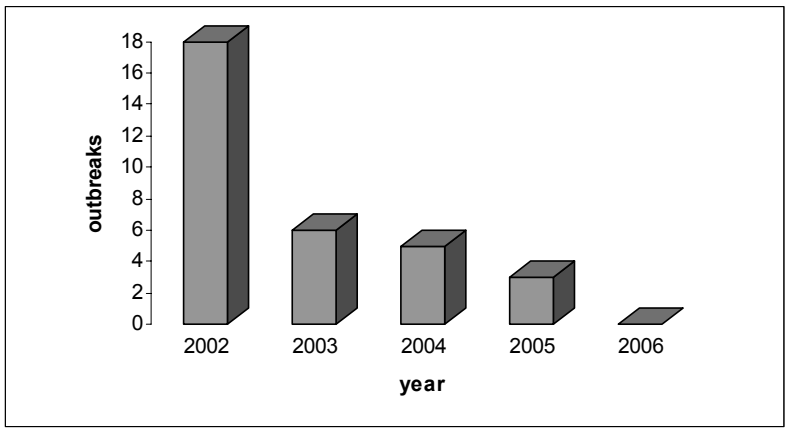

Figure 2—Number of outbreaks in Scotland.

Although in almost 90 percent of the cases the outbreaks could be linked to recent imports of host plant material, in some cases the origin of the disease remained unclear. That was the case when the disease occurred on own propagated stock or on plants imported long ago (a year or even longer). This raises the question how long the pathogen can remain in its host in a latent stage. There might also be the possibility that the pathogen is introduced to a nursery with plants that are not known to be host plants. These plants might show atypical or weak symptoms or no symptoms at all, and might therefore be overlooked by the inspectors.

Despite the positive development it is important to stay vigilant as a new outbreak in early 2007 has shown. *Phytophthora ramorum* is still widespread in Europe and as long as host material is traded there is always the danger that the disease will re-appear.

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Spatial Variation in Effects of Temperature on Phenotypic Characteristics of *Phytophthora ramorum* Isolates From Eastern Sonoma County[1]

Valerie Sherron,[2] Nathan E. Rank,[2] Michael Cohen,[2] Brian L. Anacker,[2] and Ross K. Meentemeyer[3]

Abstract

Quantifying the growth rates of plant pathogens in the laboratory can be useful for predicting rates of disease spread and impact in nature. The purpose of this study was to examine phenotypic variation among isolates of *Phytophthora ramorum* collected from a foliar host plant species, *Umbellularia californica* (California bay laurel), naturally-occurring in localities in three regions (southwest, east, northwest) in a 275 km^2 study area in Sonoma County. These regions differ topographically, in history of infection by *P. ramorum*, and in microclimate and plant community composition.

We quantified phenotypic variation among *P. ramorum* isolates among regions, plots, and individual trees, allowing us to detect host-plant effects on *P. ramorum* phenotype, and to detect geographic structure in phenotypic characteristics. It also allowed us to test the hypothesis that North American isolates of *Phytophthora ramorum* show relatively high levels of phenotypic variation despite their low levels of genetic variation.

Isolates were collected in spring 2006, after a relatively wet period favorable to *P. ramorum* growth. Growth of 37 different isolates, one or two per tree, from 25 California bay laurel trees from 15 plots was measured on V8 agar plates at four different temperatures (16, 20, 24, 26°C). Each plate was scanned five times over 12 days (day 2, 5, 7, 9, 12). Colony diameter was quantified at each time point using image analysis software (NIH Image). Change in colony diameter over time was analyzed using linear regression of diameter versus day. Growth was linear through day 12 for all replicates, allowing us to estimate growth rate using the slope from each regression. We used growth rate (slope value) and colony size (diameter at day 12), as dependent variables in analyses with region, plot, tree, and isolate as nested grouping factors that were crossed with the grouping factor of treatment temperature in a mixed model analysis of variance.

Growth patterns at 16 to 24°C showed similarities for all isolates, where growth extended horizontally along the agar surface without extending vertically. In some isolates, one or more distinct rings, indicating high densities of chlamydospores, were detected after a few days of growth. Growth at 26°C was substantially different than at the lower temperatures. In many

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cases, the filaments grew upwards away from the agar, and in others, they extended much more thinly away from the origin than observed at lower temperatures. It appeared that this temperature approached the thermal limit for *P. ramorum* under these conditions. For this reason, the 26°C treatment was excluded from further analysis.

We found significant variation in colony size over time among regions, trees within a locality, and among individual isolates. Colonies from the southwest region grew faster than those from the north and east. Growth was significantly greater at 20°C and 24°C than at 16°C. Differences among regions and trees depended on temperature, and individual isolates varied in effects of temperature on colony size.

A multiple regression analysis revealed that *P. ramorum* growth was negatively related to longitude (more rapid growth for isolates from cooler, western plots than from hotter, eastern ones) and elevation, but positively related to Topographic Moisture Index. This suggests that the effect of source environment on growth can persist over a considerable length of time (months in the laboratory).

In conclusion, we found significant variation in *P. ramorum* phenotypic characteristics across three scales within a relatively small geographic area. This high level of phenotypic plasticity may in part explain the invasion success of this pathogen. Accounting for this variation may play an important role in predicting future disease spread.

Key words: *Phytophthora ramorum*, growth rate, California bay laurel.

Environmental Parameters Affecting Inoculum Production From Lilac Leaf Pieces Infected With *Phytophthora ramorum*[1]

Nina Shishkoff[2]

Abstract

Leaves with lesions caused by *Phytophthora ramorum* Werres, de Cock & Man in't Veld often drop off infected plants. Because fallen leaves might serve as sources of inoculum both for the above-ground tissues of host plants and for their roots, this study quantified the inoculum produced by such leaves on the surface of pots when exposed to different watering regimes or different temperatures. In one experiment[3], a 6.5 cm^2 piece of infected lilac leaf was placed on the surface of potting mix in pots containing healthy lilac plants (*Syringa vulgaris* L.). The pots were then watered using a leaf hygrometer to ensure constant moist conditions, or using trickle irrigation for five minutes twice a day. Pots were incubated under greenhouse conditions. Leaf pieces were assayed at 0, 1, 2, 3, 4, 7, 10, 14, 18, and 22 days by shaking them in a known volume of sterile distilled water, then plating 0.5 ml aliquots on PARP selective media. This allowed the number of propagules produced by each leaf piece to be calculated. At the end of the experiment, four soil cores were taken from each pot and root segments in them were washed and plated on PARP media. A mixed model regression analysis for repeated measures over time was run on the first five data points, showing that propagule production declined over time for the first four d (P=.0001) from approx. 120,000 sporangia/leaf piece at time 0 to almost nothing by day 4, but declined significantly less steeply under the constantly moist conditions (P=.0009). After the first two weeks or so, the leaves in the overhead misted treatment began to give off a new pulse of propagules; examination of plates showed that initially, propagules were predominantly sporangia, but as the leaf decayed, sporangia were replaced by chlamydospores released from disintegrating tissue. When lilac plants in the pots were examined at the end of a month, there was one infected leaf observed in the moist treatment in one replicate. Overall, 28 percent of plants exposed under moist conditions developed root infections, while only 6 percent of plants exposed to trickle irrigation did; a chi-square test comparing infection by treatment showed this to be significant at P =0.07. However, these infections were very slight: in infected plants, only .05 to 3.8 percent of root segments plated yielded colonies of *P. ramorum*. In another experiment[3], infected leaf pieces were placed on the surface of potting mix in pots containing healthy lilac plants and watered using overhead irrigation for 5 minutes 1, 2, or 3 times a day. Leaf pieces were assayed at 0, 1, 2, 3, 4, 5, 7, 10 and 14 days. After a month, root samples were taken. A mixed model regression analysis was run on the first five data points, showing that propagule production declined with time (P<0.0001) but declined significantly slower in

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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[3] Preliminary results from these experiments were presented in: Shishkoff, N. 2006. Behavior of lilac leaves infected with *Phytophthora ramorum* when placed on the surface of nursery pots. Phytopathology. 96: S107.

pots watered three times a day (P<.001). Infected roots were observed in all treatments (5 percent in pots watered once a day, 10 percent when watered twice a day, and 20 percent when watered three times a day), but a chi-square test to see if watering frequency had an effect of root infection was not significant. Again, the actual number of infected roots detected was very low, from 0.6 to 1.8 percent. In a third experiment[3] 0, 2, 4, 8, or 16 leaf pieces were placed on the surface of soil kept moist with overhead misting or watered using trickle irrigation twice a day. After a month, soil cores were taken from each pot and root segments in them plated on PARP media. No root infection was seen in pots with trickle irrigation. Root infection was observed in pots kept constantly moist, from 38 percent in pots with 2 to 4 leaf pieces, 62 percent in pots with 8 leaf pieces, and 75 percent in pots with 16 leaf pieces. A chi-square test to see if number of leaf pieces influenced frequency of root infection was significant at P= 0.02. As with previous experiments, the actual percent root infection observed in these infected plants was low: from 0.6-13.6 percent. In a fourth experiment, the effect of temperature was studied. Infected leaf pieces were placed on the surface of potting mix in pots containing healthy plants kept in controlled-environment chambers set at 10°C, 15°C, 20°C, and 25°C and trickle-irrigated twice daily. The leaf pieces were removed for sampling at 0, 1, 2, 3, and 4 days. Practically no propagules were seen after day one. An analysis of variance was run on data from day one, showing significantly more propagules produced from leaf pieces at 10°C. Because it is difficult to distinguish the effects of temperature from moisture, infected leaf pieces were also placed in vials of sterile distilled water in controlled-environment chambers set at 10°C, 15°C, 20°C, and 25°C. The leaf pieces were assayed at 0, 1, 2, 3, and 4 d. Different propagules were observed at different temperatures. At 10 or 15°C, propagules were predominantly zoospores, while at 20 or 25°C, they were predominantly sporangia. An analysis of variance done at day 1, 2, 3 and 4 showed that there were greater numbers of propagules recovered from 10 and 15°C treatments than from 20 and 25°C treatments. It was impossible, from these data, to separate an effect of temperature on sporangial production from one on zoospore production. These results confirm the importance of fallen leaves as inoculum producers under greenhouse conditions and also confirm that cool moist conditions are more conducive to inoculum production that warm dry conditions. Although lilac roots did become infected from exposure to these fallen leaves, the amount of infection was very slight after 1 month under greenhouse conditions. Root infections have been shown to spread to above-ground plant parts in *Rhododendron* (Lewis and others 2004), so they may be significant in the disease cycle even in low amounts. Inoculum produced from fallen leaves might also be spread in irrigation splash and runoff.

Key words: *Phytophthora ramorum, Syringa vulgaris,* lilac.

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The Maturation and Germination of *Phytophthora ramorum* Chlamydospores[1]

Aaron L. Smith[2] and Everett M. Hansen[3]

Abstract

Chlamydospores are a distinctive feature of *Phytophthora ramorum*. They are formed quickly in agar, and within colonized leaves. We followed their development and maturation *in vitro* and *in vivo*, and studied conditions affecting their germination. Cell walls of mature *P. ramorum* chlamydospores are thicker than reported for other *Phytophthora* species, although thin-walled chlamydospores are also formed. Chlamydospores formed within rhododendron leaves are smaller with thicker walls than spores formed *in vitro*. Chlamydospore development begins on hyphae less than two days old, and chlamydospores reach maximum size in about 10 days. Chlamydospores are formed continuously as *P. ramorum* develops, so spores of all ages are mixed in a colony. Chlamydospores of *P. ramorum* germinate *in vitro* at a low but highly variable frequency. In our experiments, the maximum germination obtained was about 13 percent, and the overall average was closer to 3 percent. Germination was higher on V8 agar than on cornmeal agar, and lowest on water agar. Smaller chlamydospores germinated more frequently than larger spores.

Key words: *Phytophthora ramorum*, chlamydospores, germination.

Introduction

Chlamydospores are a distinctive feature of *Phytophthora ramorum*. They are formed quickly in agar and in V8 juice broth (V8JB). In other *Phytophthora* species, chlamydospores have allowed the organism to survive periods of natural environmental extremes such as: temperature, microbial antagonism, and the absence of host tissue. When conditions conducive to vegetative growth are encountered, the chlamydospore can germinate and create new colonies vegetatively or through the production of sporangia and subsequent release of zoospores. For many species of *Phytophthora,* the chlamydospore is an important survival mechanism that is not well understood. We followed chlamydospore development and maturation *in vitro* and studied conditions affecting their germination.

Materials and Methods

Wall thickness and diameter development—Chlamydospores were grown in V8 and V8JB cultures for 2 to 120 days. Chlamydospore wall thickness and diameter were measured at 1000x under bright field illumination.

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Chlamydospore germination—Chlamydospores were grown for 10 days in 25 ml V8JB. V8JB cultures rinsed of medium were rinsed three times for 30 seconds with ionized, distilled water on a 100 µm sieve, the rinsate was diluted to 400 ml. Then 100 ml of chlamydospore suspension was filtered through a 5 µm polycarbonate filter. Filters were placed face-down on the surface of V8, cornmeal with antibiotics (CAR), and water agar plates and peeled off. Petri plates were sealed and incubated for 24 hours at 20°C in the dark. The number of germinated and intact chlamydospores was tallied in multiple 100x fields of view. Percent chlamydospore germination was calculated by the formula: percent germination = (number germinated chlamydospores) / (number germinated chlamydospores + number intact non-germinated chlamydospores).

Results and Discussion

Chlamydospore maturation in V8JB—Mean chlamydospore wall thickness increased from 1.8 µm in 10-day-old age class chlamydospores to an average of 2.3 µm in chlamydospores in the 20 to 120 day old age classes. Mean maximum chlamydospore wall thickness continued to increase from 2.7 µm in the 10-day-old age class to 4.0 µm in the 90- and 120-day-old age classes. Mean diameter of chlamydospores formed in V8JB averaged 53.2 µm in 10- to 120- day age classes. This pattern was repeated in mean maximum chlamydospore diameter values that averaged 71.6 µm in chlamydospores 10 to 120 days old.

Relationship between chlamydospore diameter and wall thickness— Chlamydospore diameter increased with wall thickness in chlamydospores grown on V8 agar for two to 31 days and in chlamydospores grown in V8JB for 10 to 120 days (fig. 1*).* The r-values for V8 agar and V8JB-grown chlamydospores were 0.69 and 0.60 respectively.

Chlamydospore germination—Chlamydospore germination began within 24 hours of plating on agar. Chlamydospores germinated to form mycelial colonies, or directly to sporangiophores and sporangia (fig. 2). The effect of nutrients on chlamydospore germination was compared in three experiments (fig. 3). In each experiment, germination was highest on V8 agar, and lowest on water agar.

Germinated and V8JB chlamydospores—Germinated mean chlamydospore wall thickness was thinner at 1.6 µm than parent V8JB culture mean chlamydospore wall thickness at 1.8 µm. Germinated mean chlamydospore diameter was smaller at 42.7 +/- 1.0 µm than parent V8JB culture mean chlamydospore diameter at 54.6 +/- 1.0 µm (Data not shown).

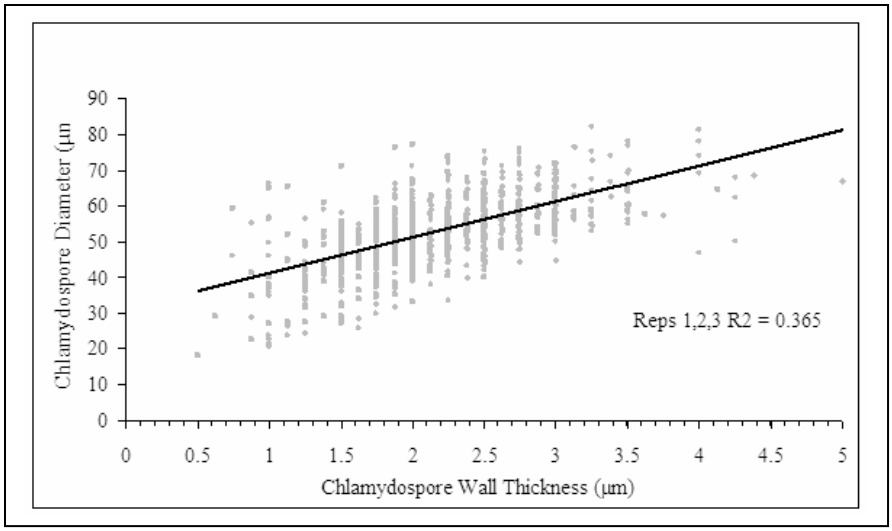

Figure 1—Diameter vs. wall thickness relationship in V8JB-grown chlamydospores aged 10 to 120 Days.

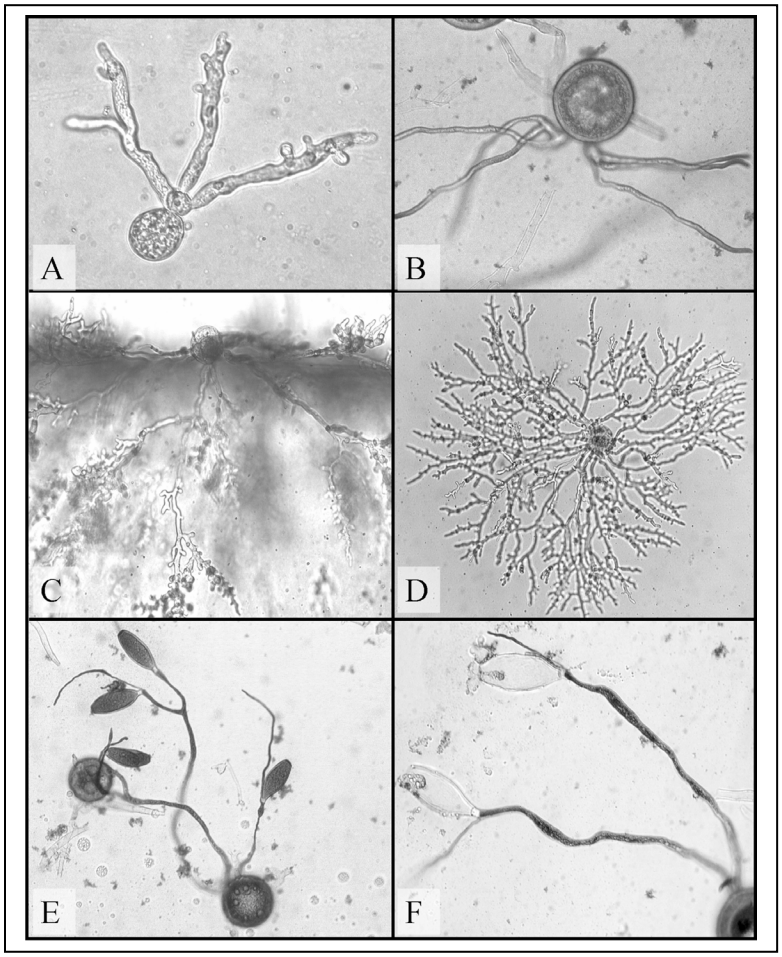

Figure 2—Chlamydospore germination after 24 hours.

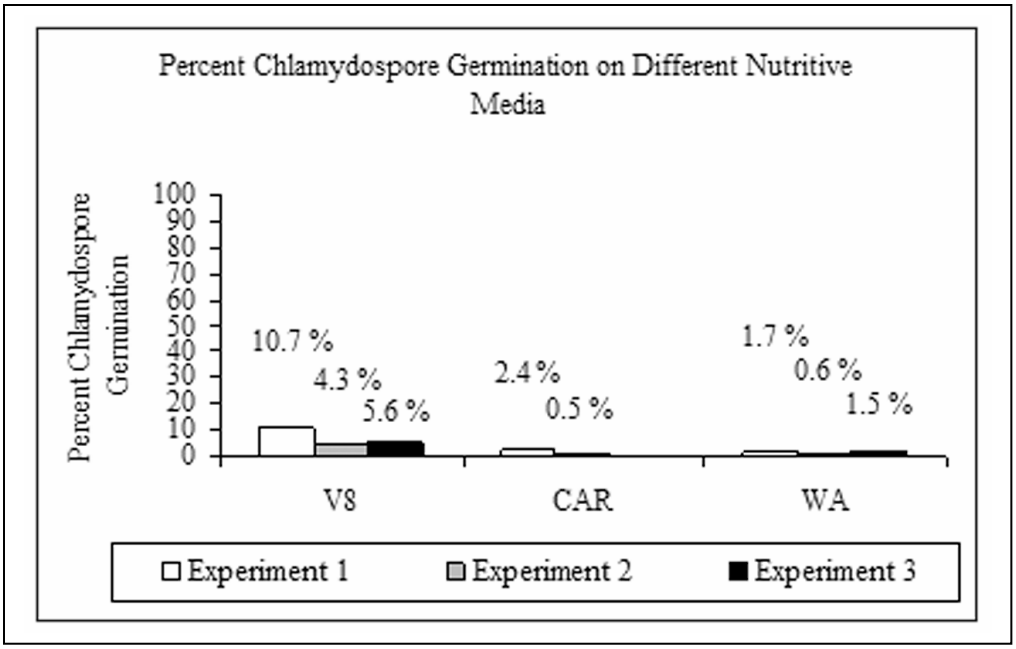

Figure 3—Chlamydospore germination on V8 agar, cornmeal agar with antibiotics, and water agar.

Conclusions

- The chlamydospore wall of *P. ramorum* is thicker than most species of *Phytophthora*.
- Most chlamydospores of *P. ramorum* were fully developed within 10 days, though subsequent wall thickening occurred up to 120 days in V8JB.
- Chlamydospore wall thickness increased with spore diameter.
- Chlamydospores with thinner walls and smaller diameters were more likely to germinate.
- The rate of germination of *P. ramorum* chlamydospores is low and variable compared with published chlamydospore germination rates of other *Phytophthora* species.
- *P. ramorum* chlamydospore germination is controlled, at least in part, by the presence of exogenous nutrients.

These results represent an important first step in understanding the basic biology of the chlamydospore in *P. ramorum,* but further research is necessary to better understand their true biological significance to the organism.

Microclimate Environmental Parameters Indexed for Sudden Oak Death in Georgia and South Carolina[1]

Pauline Spaine,[2] William J. Otrosina,[2] Stanley J. Zarnoch,[3] and Sharon V. Lumpkin[2]

Abstract

We monitored Ericaceous habitat in Georgia and South Carolina for temperature, dew point and humidity ranges throughout a two year period. Temperature and humidity data were used to characterize their range in Georgia and South Carolina where potential SOD susceptible hosts occur. This data suggests risk for SOD development may be more widespread in southeastern forest microclimates than current risk maps indicate. A sudden oak death infection risk index based on laboratory infection parameters for *Phytophthora ramorum* was developed for use in the field. In addition, we monitored streams and bleeding bark cankers from different ecotypes throughout Georgia using various methods for detection of *Phytophthora* propagules. Samples cultured from bleeding cankers on oaks yielded a broad assortment of organisms. Most often found associated with these cankers are the fungal genera *Mortierella* and the potential antagonist *Trichoderma.*

Key words: Temperature, humidity, leaf wetness.

Introduction

Since 1995, large numbers of tanoaks and oaks have been dying in the coastal counties of California. The death of these trees has been attributed to the Oomycete or water mold *Phytophthora ramorum,* the causal agent of the disease commonly called sudden oak death (SOD). The potential *P. ramorum* susceptible plant host list has been rapidly expanding as a result of pathological data obtained through controlled environmental testing.

Risk maps prepared by the United States Department of Agriculture Forest Service (USDA-FS), Forest Health Monitoring and the Animal and Plant Health Inspection Service (APHIS) have outlined some of the risk parameters associated with this pathogen, but there are large information gaps on the interaction of environmental conditions necessary for infection. Early prediction/risk analysis maps for SOD, based upon available data on susceptible host occurrence, contained large areas of no risk in the piedmont and coastal areas of South Carolina and Georgia.

Although the region contains many potential hosts for *P. ramorum*, much of north Florida, the Georgia piedmont, and the Georgia coastal plain, throughout much of the year are regarded as low risk areas for SOD. Based upon generalized environmental

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data, the regional environment in the southeastern United States has not been regarded as conducive for the pathogen based upon our current knowledge of its biology (W. Smith, Research Triangle Park, Raleigh, NC, personal communication, author of the Forest Health Monitoring risk map for *P. ramorum*). On the other hand, the diverse landscapes, vegetative cover types, and abundant waterways in this region can encompass a considerable number of sites where environmental conditions are conducive to *P. ramorum* infection.

In spring 2004, *P. ramorum* infected nursery stock was shipped from California to many eastern states, including Georgia and South Carolina. Over 80 locations in Georgia have confirmed the presence of *P. ramorum* infections on nursery stock. Some plants were sold to the public before the nursery stock could be quarantined. In the southeastern United States, many susceptible plant species occur where potentially optimal temperature and humidity for *P. ramorum* infection may exist for suitable periods. These optimal conditions may be present outside the areas indicated on current risk maps. *P. ramorum* has a wide host range that includes eastern oaks and Ericaceous plants species, both of which are abundant in southeastern forests (Tooley and Kyde 2007). Many genera of east coast plants can support *P. ramorum* sporulation, potentially infecting the ecologically and economically important eastern oaks. Since economically important tree species are at risk, coupled with the large-scale resolution of current risk maps, we conducted this study to identify potential critical microclimatic variables conducive for *P. ramorum* infection. We also sampled streams, other waterways, and tree wound effluxes for the presence of *P. ramorum* and other *Phytophthora* species in areas having susceptible vegetation. Information gained from this study will strengthen current SOD risk maps and models by developing an SOD susceptibility index. Such an index can identify where monitoring resources could be more efficiently deployed.

Materials and Methods

HOBO H8 Pro-Series® data loggers were placed in high SOD risk areas according to the APHIS SOD risk map for Georgia and South Carolina. The data loggers were placed in areas that supported *P. ramorum*-susceptible vegetation. These recorders collected the temperature, humidity and dew point at the location every hour of the day. Our *P. ramorum* infection index was formulated as a function of the dew point being equal to the optimum temperature (± 0.5°C) for infection (between 18 and 22 °C), for greater than or equal to six consecutive hours in a day (Garbelotto and others 2003). These criteria theoretically indicate free water presence on surfaces when the temperature and dew point conditions are met. Data were collected throughout the entire two years and expressed graphically when the criteria were met, but criteria for infection index was only met in those months present on the graph.

A water baiting technique was employed to sample streams for *Phytophthora* species in areas of SOD susceptible vegetation. Using a generic portable submersible pump, an eight foot piece of flexible plastic tubing was taped to a 3.05 m (10 ft) pole affixed to the outlet end of the pump. A brass sieve of 300 to 400 µ was fixed to the bottom of the intake to prevent large debris from entering the pump. This apparatus was then suspended out into the river where 8 to 12 l of water were collected and placed in several Erlenmeyer flasks. This water was then filtered through 20 and 30 µMillipore filters (Hong and Kong 2002) using a hand held vacuum pump. The filters were kept

cold and transported back to the lab within one hour of collection. The contents of the Millipore® filters were then washed off with 10 to 15 ml of sterile distilled water from a sterile syringe and plated onto PARP plus hymexazole medium (Ferguson and Jeffers 1999) in 100 µl drops on five spots per plate.

Additional isolations were conducted on effluxes from the bark of white and red oaks. Pieces of the wet bark samples were cultured on modified PARP medium (Ferguson and Jeffers 1999).

Results and Discussion

We developed an index of *P. ramorum* infection risk parameters for microsites based on research done on the physiology of *P. ramorum* infection parameters (Garbelotto and others 2003). Data shown represent two years analysis of infection index values at the four locations (fig. 1). The bars represent the infection index expressed as number of days with six or more hours of leaf wetness occurrence at the optimum temperatures for *P. ramorum* infection (Garbelotto and others 2003).

In 2005, our *P. ramorum* infection susceptibility index revealed that the Brunswick, GA (elevation = 11 m) coastal site was bimodal (fig.1), with infection indices ranging from May to January, with a peak in September. Sites in Atlanta (elevation = 266 m) had infection indices from May to October, with a peak in June (fig. 1). A site at Chatsworth, GA (elevation = 250 m) also had infection indices from May through

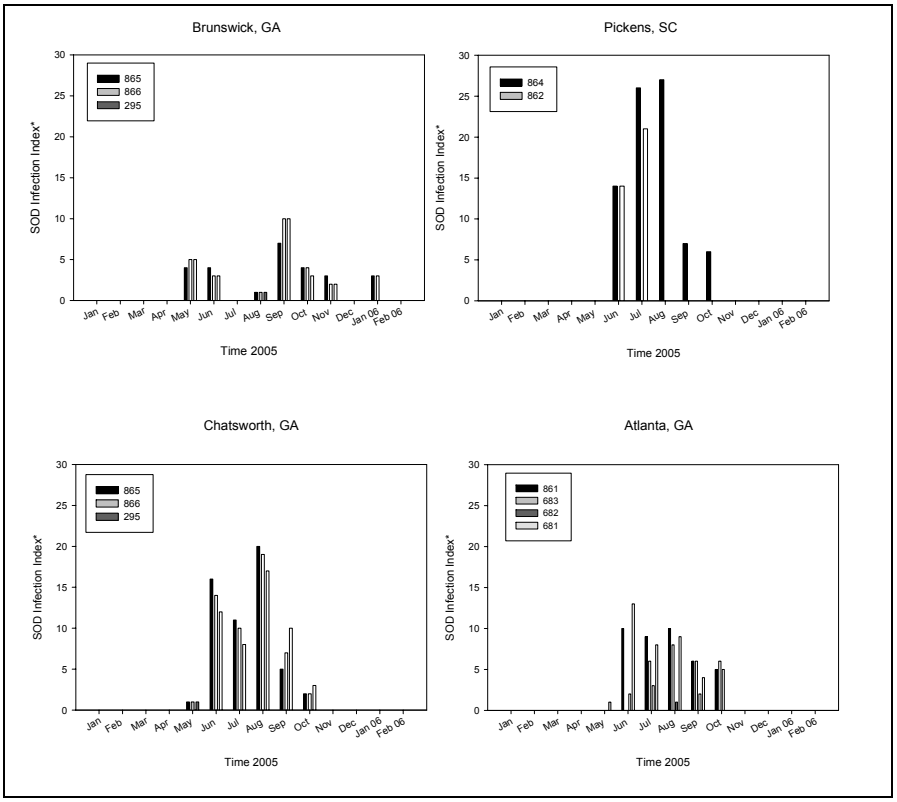

Figure 1—*Phytophthora ramorum* infection index for number of days when infection criteria are met during 2005-2006. Legend key represents specific HOBO® data collectors.

October, but the peak index value was in August (fig. 1). Our highest elevation site, Pickens, SC (elevation = 362 m), had the highest number of infection index days during July and August, but had the narrowest infection index range, extending only from June through October (fig. 1). Our microsite specific temperature and humidity data showed greater SOD risk in the Georgia coastal environments and western South Carolina than the USDA-FS (Smith and others 2002) or APHIS risk maps (Fowler and others 2005). Mid-level elevations such as Chatsworth have the same amount of risk as coastal Brunswick, GA in the month of September. Pickens, Chatsworth and Atlanta areas had extremely high infection indices for the month of August, greater than indicated by the APHIS risk map. The infection index was present for a greater number of months in Brunswick, Georgia probably due to high humidity along the coast throughout much of the year. Many genera of east coast plants could support *P. ramorum* sporulation, thus potentially impacting the ecologically and economically important eastern oak species.

In 2006, our monitored sites continued to have similar infection index values (fig 2). Generally warmer temperatures and higher humidity throughout the year contributed to the infection index values appearing earlier in the spring and later into the fall months at the Brunswick site. Overall, Brunswick microsites had up to 10 index days over a period of 10 months. The number of months yielding index days in Brunswick was at least four months longer compared to the three other locations. Based upon the results of our infection index, *P. ramorum* can become established along the southeastern coastline as it has along the northern California coastal region.

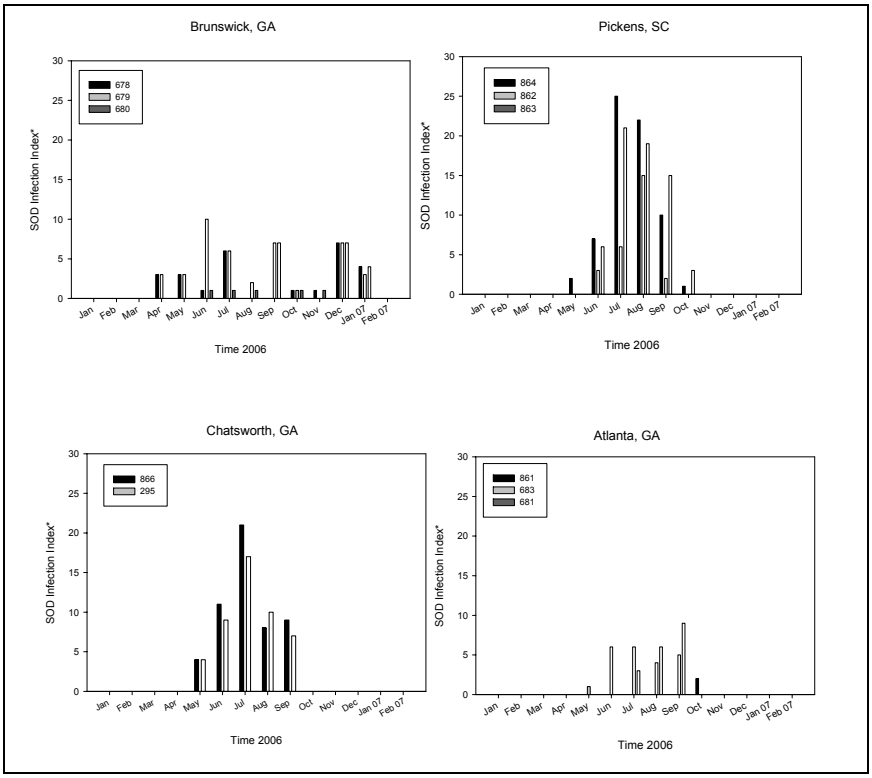

Figure 2—*Phytophthora ramorum* infection index for number of days when infection criteria are met during 2006-2007. Legend key represents specific HOBO® data collectors.

Also in 2006, Chatsworth, GA and Pickens SC had the greatest number of days that met the infection index criteria from May through September and May through October, respectively (fig. 2), indicating that microclimate in these locations was suitable for infection. The infection index had some variation among monitoring devices within a location, indicating the importance of considering microclimate conditions in assessing risk.

In Atlanta, the number of infection index days in 2006 was nine or less for any particular month (fig 1). In general, the Atlanta sites are not buffered by large areas of vegetation to modify temperature extremes. The contrast between Atlanta and the three more rural environments we monitored may have been due to the urban heat island effect, where higher temperatures reduced the number of days falling within the infection index criteria.

Overall, our index could be used to monitor locations for SOD disease risk in combination with vegetation maps. The infection index may also be valuable in the early stages of *P. ramorum* introductions and detection in forests. Dirac and Menge (2003) found that the infection by *Phytophthora* sp. was dependent on carbohydrate flow in the roots of host plants. The flow of carbohydrates generally corresponds to warmer temperatures conducive for plant growth. Many genera of susceptible east coast plants could become infected with *P. ramorum* during the warmer months, as shown by our data. Our infection index could be used as a risk assessment tool in ecologically important eastern oak microclimatic areas that support *P. ramorum* susceptible understory vegetation. This would be particularly important when understory plants are infected but overstory trees are not yet symptomatic. Resources can then be directed to areas where moisture and temperature conditions for infection of at risk tree species are probable. On the other hand, once infection is established within the host, there is no information on how *P. ramorum* is affected by local environmental conditions. Such information can only be gathered from field research on important host species such as the eastern oaks.

Sampling water from streams in and around the piedmont in Georgia yielded several *Phytophthora* species, but no presence of *P. ramorum*. Our methodology of high volume surface water sampling (8 to 12 l per location) may enhance early detection of *P. ramorum* by increasing the probability of encountering its propagules in water ways. Species commonly found were identified as *P. cinnamomi* Rands, *P. cryptogea* Pethybr. et. Laff., and *P. nicotianae* Breda de Haan. Sampling and isolations from wet bark effluent consistently yielded genera such as *Mortierella* sp., *Fusarium* sp., and *Tricoderma* sp., with *Moriterella* as the predominate genus found in these effluxes. Continued study of the mycoflora associated with potential *P. ramorum* infection sites is important for finding possible antagonists to this economically significant pathogen.

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Comparing *Phytophthora ramorum* Diagnostic Protocols for the National Sudden Oak Death Stream Monitoring Program[1]

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Abstract

Oregon was a participant in the pilot test of the national stream monitoring protocol for SOD. We routinely and continuously monitor about 50 streams in and near the SOD quarantine area in southwest Oregon using foliage baits. For the national protocol, we added six additional streams beyond the area of known infestation, and compared results from different diagnostic tests with results from six streams within the quarantine area. Foliage baits were in streams for two weeks, then assayed by: 1) culture on selective medium; 2) PCR diagnosis using the P. lat multiplex primers; 3) nested PCR using the Garbelotto and others protocol; and 4) Real-Time PCR using the CSL protocol. Most samples at most sample times gave consistent results, regardless of diagnostic method. However, occasional positive results from the various molecular protocols were not supported by isolation in culture, and despite intensive surveys near the streams, no plants infected by *P. ramorum* were located. We concluded that culturing from leaf baits was the single most reliable diagnostic method. False positives arose from several sources, including laboratory error, insufficient specificity of primers, and presence of undescribed *Phytophthora* species in the streams.

Key words: *Phytophthora ramorum*, sudden oak death, PCR, stream monitoring.

Introduction

Oregon was a participant in the 2006 pilot survey for *Phytophthora ramorum* in forest streams in the United States, coordinated by Steve Oak of the United States Department of Agriculture (USDA)-Forest Service. The objective of the pilot survey was to develop and modify a standard stream baiting and diagnostic protocol for use across the country. Oregon routinely and continuously monitors about 50 streams in and near the sudden oak death (SOD) quarantine area in southwest Oregon using rhododendron and tanoak leaf baits. For the national pilot survey, we added six additional streams beyond the area of known infestation, and compared results from different diagnostic tests with results from six streams within the quarantine area (fig.1 and table 1). Foliage baits in nylon mesh bags were in streams for 2 weeks, then assayed using four diagnostic techniques.

Isolation on Selective Medium

Lesions and petioles of stream bait leaves were plated on CARP+BH and later examined for *P. ramorum*.

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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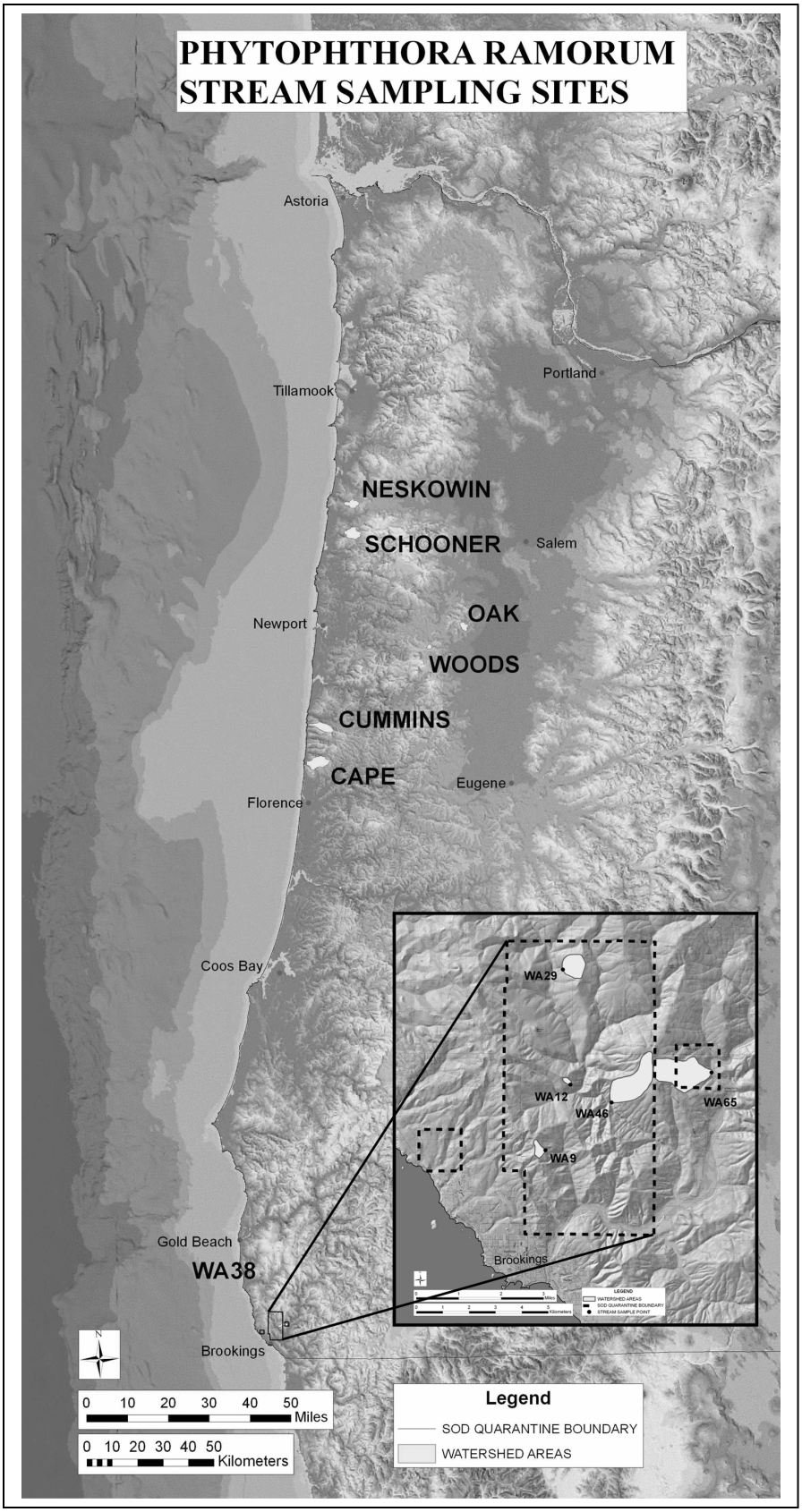

Figure 1—Map of Southwest Oregon emphasizing monitored watersheds and 2006 quarantine boundaries.

Table 1—Site and vegetation information for the 12 Oregon streams selected for the 2006 pilot survey of the National Stream Monitoring Protocol for SOD

STREAM	LOCATION	WATERSHED AREA (hectares)	VEGETATION TYPE	PREDOMINANT TREE SPECIES	DISTANCE TO KNOWN P. RAMORUM OCCURRENCE (Nursery or Forest) (kilometers)	P. RAMORUM HISTORY
WA9	Curry Co.	18	Tanoak-Douglas-fir forest (50%); Douglas-fir (50%)	Tanoak, Douglas-fir	0	Culture and PCR positive since 2002
WA12	Curry Co.	6	Tanoak-Douglas-fir forest	Tanoak, Douglas-fir	0	Culture and PCR positive since 2002
WA38	Curry Co.	16	Tanoak forest (50%); Douglas-fir (50%)	Tanoak, Douglas-fir	17	Ramorum never confirmed
WA46	Curry Co.	169	Tanoak-Douglas-fir forest	Tanoak, Douglas-fir	0	PCR + since 2004, Infected tanoak found in 2005
WA65	Curry Co.	194	Redwood-Douglas-fir-tanoak forest (30%); tanoak-Douglas-fir (70%)	Tanoak, Douglas-fir, redwood	0	Culture and PCR positive since 2005, infected tanoak 2006
WA29-2	Curry Co.	64	Tanoak-Douglas-fir forest	Tanoak	1	Culture and PCR positive in 2006, source plant never found
Cape	Lane Co.	3,094	Douglas-fir-hemlock-red cedar forest (50%); Spruce-hemlock (40%); mixed conifer-deciduous (10%)	Douglas-fir, hemlock, alder	30	Ramorum never confirmed
Cummins	Lane Co	2,131	Douglas-fir-hemlock-red cedar forest (60%); Spruce-hemlock (30%); mixed conifer-deciduous (10%)	Douglas-fir, hemlock, alder	15	Ramorum never confirmed
Neskowin	Tillamook Co.	1,290	Douglas-fir-hemlock-red cedar forest (70%); mixed conifer-deciduous (30%)	Douglas-fir	38	Ramorum never confirmed
Oak	Benton Co.	773	Douglas-fir-hemlock-red cedar forest (75%); mixed conifer-deciduous (20%)	Douglas-fir	38	Ramorum never confirmed
Schooner	Lincoln Co.	2,121	Douglas-fir-hemlock-red cedar forest (60%); mixed conifer-deciduous (40%)	Douglas-fir, hemlock, alder	25	Ramorum never confirmed
Woods	Benton Co.	1,000	Douglas-fir-hemlock-red cedar forest	Douglas-fir	50	Ramorum never confirmed

Phytophthora lateralis Multiplex PCR Assay

The *P. lateralis* (*P.lat*) multiplex primers were developed as a species specific diagnostic for *P. lateralis* before *P. ramorum* was discovered (Winton and Hansen 2001). The assay has one set of primers for *P. lateralis* (amplification indicating the presence of *P. lateralis*), and a second set that amplifies plant DNA (amplification indicating a successful extraction and PCR reaction). Because of the similarity of the *P. lateralis* and *P. ramorum* sequences in the target region, the *P. lateralis* primers also consistently amplify *P. ramorum* DNA.

Nested PCR Assay

The nested protocol, published by Garbelotto and others (2002)., was developed for *P. ramorum* and has the advantage of two amplification steps, aiding in the detection of low DNA copy samples. In round one the first set of primers is *P. ramorum* specific, bracketing 5.8S, and amplifying portions of both ITS1 and ITS2. The round two reaction primers target a portion of ITS2 that falls within the round one amplicon and uses the diluted product of round one for template.

CSL Real-time PCR Assay

The CSL protocol was developed as a real-time *P. ramorum* specific PCR assay (Tomlinson and others 2005, Hughes 2003). *Phytophthora ramorum* specific primers and labeled probe, along with primers and probe designed to amplify plant DNA, are run together. Using this technique eliminates the need to visualize results on a gel.

Materials and Methods

Rhododendron and tanoak leaf baits were washed and examined for lesions or other evidence of infection after a two week baiting period. Petioles and lesions were excised and each piece was cut in two, half being plated for isolation onCARP+BH and half being extracted with a CTAB – modified Qiagen DNeasy Tissue Kit extraction. The same DNA extract was used for all three PCR assays.

The selective medium CARP+BH was made using 17grams of BBL Corn Meal Agar and 1 liter of de-ionized water. After autoclaving and cooling, the medium was amended with 20 ppm Delocid (50 percent natamycin salt), 200 ppm Ampicillin

sodium salt, 10 ppm Rifamycin SV sodium salt, 30 ppm Benlate (benomyl 50WP) and 25 ppm Hymexazol (99 percent).

Isolation plates were incubated at 20°C. Plates were examined at approximately days 3 and 7 for *P. ramorum* and other *Phytophthora* species. All *Phytophthoras* were transferred to CARP for further clean up and eventually to corn meal agar augmented with beta sitosterol.

The *P.lat* multiplex PCR was performed in 15 µL reactions of 1X enzyme buffer, 200 µM dNTP, 0.4 µM PLITS87F and PLITS786R (*P. lateralis* primers), 0.07 µM NS1 and NS2 (universal primers), 0.15 µL 5 percent blocking powder, 0.8 U RedTaq DNA polymerase, and 2 µL DNA template. The thermal cycler conditions were 2 minutes at 94°C, 35 cycles of 94°C for 30 seconds, 52°C for 30 seconds and 72°C for 1 minute, followed by 72°C for 4 minutes and 24°C for 1 minute. The products were visualized with ethidium bromide under UV light in a 1.5 percent agarose gel.

The nested PCR round one was performed in 25 µL reactions of 1X enzyme buffer, 200 µM dNTP, 0.4 µM Phyto1 and Phyto4, 0.5 µL 5 percent blocking powder, 1.25 U RedTaq DNA polymerase, and 2 µL DNA template. Round two used the same reaction cocktail replacing Phyto1 and 4 with Phyto2 and Phyto3, and adding 2 µL of round one PCR product diluted 1:10. The thermal cycler conditions were 85 seconds at 94°C, 34 cycles of 93°C for 35 seconds, 62°C for 55 seconds and 72°C for 50 seconds + 5 seconds/cycle, followed by 72°C for 10 minutes. The products were visualized with ethidium bromide under UV light in a 1.5 percent agarose gel.

The CSL real-time PCR was performed in 25 µL reactions of 1X Taqman Universal master mix, 0.0 5uM Pram 114-FC, 0.2 µM Pram 1527-190R 0.1 µM Probe 1527-134T (for *P. ramorum*), 0.1 µM COX-F, COX-RW and Probe COX (universal), 2.375 µL 25 percent Trehalose and 2 µL DNA template. The thermal cycler conditions were 10 minutes at 94°C followed by 45 cycles of 94°C for 15 seconds, 60°C for 1 minute, 72°C for 1 minute and a plate read. The threshold was set at 10 standard deviations above the mean fluorescence of cycles 3 to15. A Ct value of less than 40 cycles was considered positive, 40 thru 45 inconclusive and anything not crossing the threshold negative.

Results and Discussion

The three molecular diagnostic assays gave consistent, positive results (table 2) in known infested streams from which *P. ramorum* is regularly cultured (WA9, WA12 and WA29-2). *P. lat* multiplex PCR and CSL real-time assays also gave consistent negative results for the streams beyond the known range of SOD in Oregon forests and from which *P. ramorum* has never been cultured (Cape, Cummins, Neskowin, Oak, Schooner, Woods and WA38).

Results from *P. lat* multiplex PCR matched epidemiological evidence in all 12 streams.

Nested PCR matched 9 of 12 streams, giving round two positives in three streams not believed to be *P. ramorum* infested, possibly due to lowered specificity in the second round reaction. CSL real-time matched for 10 of 12 streams, although these results vary from sample to sample in recently infested streams with low inoculum loads.

Sites	Collection	Culture	P.lat multiplex	Nested round 1	Nested round 2	CSL - Real-time
Cape	080906	Phsp	neg	neg	neg	neg
Cape	082106	Phsp	neg	neg	neg	neg
Cape	090706	Phsp	neg	neg	neg	neg
Cape	092106	Phsp	neg	neg	neg	neg
Cape	100506	Phsp	neg	neg	neg	neg
Cape	101806	Phsp	neg	neg	neg	neg
Cape	110206	Phsp	neg	neg	neg	neg
Cummins	080906	M	M	M	M	M
Cummins	082106	Phsp	neg	neg	neg	failed
Cummins	090706	Phsp	neg	neg	neg	neg
Cummins	092106	Phsp	neg	neg		neg
Cummins	100506	Phsp	neg	neg		neg
Cummins	101806	Phsp	neg	neg	neg	neg
Cummins	110206	Phsp	neg	neg		neg
Neskowin	080906	Phsp	neg	neg	neg	neg
Neskowin	082106	Phsp	neg	neg	neg	neg
Neskowin	090706	Phsp	neg	neg	neg	neg
Neskowin	092106	Phsp	neg	neg	neg	neg
Neskowin	100506	Phsp	neg	neg	neg	neg
Neskowin	101806	Phsp	neg	neg	neg	neg
Neskowin	110206	Phsp	neg	neg		neg
Oak	080906	Phsp	neg	neg	neg	neg
Oak	082106	Phsp	neg	neg	neg	neg
Oak	090706	Phsp	neg	neg	neg	neg
Oak	092106	Phsp	neg	neg	neg	neg
Oak	100506	Phsp	neg	neg	neg	neg
Oak	101806	Phsp	neg	neg	neg	neg
Oak	110206	Phsp	neg	neg	neg	neg
Schooner	080906	Phsp	neg	neg	neg	neg
Schooner	082106	Phsp	neg	neg	neg	neg
Schooner	090706	Phsp	neg	neg		neg
Schooner	092106	Phsp	neg	neg	neg	neg
Schooner	100506	Phsp	neg	neg	neg	neg
Schooner	101806	Phsp	neg	neg	neg	neg
Schooner	110206	Phsp	neg	neg	neg	neg
Woods	080906	Phsp	neg	neg	neg	neg
Woods	082106	Phsp	neg	neg	neg	neg
Woods	090706	Phsp	neg	neg	neg	neg
Woods	092106	Phsp	neg	neg	neg	neg
Woods	100506	Phsp	neg	neg	neg	neg
Woods	101806	Phsp	neg	neg	neg	neg
Woods	110206	Phsp	neg	neg	neg	neg
WA9	080706	M				
WA9	082106	M				neg
WA9	090506					40.03
WA9	091806					
WA9	100206	phsp				
WA9	101506	phsp				
WA9	102906					
WA12	080706	M	neg	neg	neg	neg
WA12	082106	M				
WA12	090506					
WA12	091806					
WA12	100206	phsp				neg
WA12	101506					
WA12	102906					
WA29-2	080706					
WA29-2	082106	phsp				
WA29-2	090506	phsp				
WA29-2	091806	phsp				
WA29-2	100206	phsp				43.7
WA29-2	101506	phsp				neg
WA29-2	102906	phsp				
WA38	080706	phsp	neg	neg	neg	neg
WA38	082106	phsp	neg	neg		neg
WA38	090506	phsp	neg	neg	neg	neg
WA38	091806	phsp	neg	neg		neg
WA38	100206	phsp	neg	neg	neg	neg
WA38	101506	phsp	neg	neg	neg	neg
WA38	102906	phsp	neg	neg	neg	neg
WA46	080706	phsp	neg	neg		neg
WA46	082106	phsp	neg	neg		neg
WA46	090506	phsp	neg	neg		neg
WA46	091806	phsp	neg	neg	neg	neg
WA46	100206	phsp	neg	neg	neg	neg
WA46	101506	phsp	neg	neg		neg
WA46	102906	phsp				42.36
WA65	080706	M				40.52
WA65	082106	M	neg	neg	neg	neg
WA65	090506	phsp	neg	neg		neg
WA65	091806	phsp	neg	neg		neg
WA65	100206	phsp				neg
WA65	101506	phsp				neg
WA65	102906	phsp				neg

Table 2—Results for seven baiting periods on 12 streams. Streams WA9, WA12, WA29-2 and WA65 were all culture positive sites previously and/or during this monitoring period. WA38 has been PCR positive three times over the course of 3 years and WA46 is frequently PCR positive with a known inoculum source although we have not yet cultured it from stream baits. Black = tested positive, neg = tested negative, phsp = other *Phytophthora* species were cultured, M and failed = missing data and gray = Ct values >40 which are inconclusive.

The advantage of a culture positive is it is an unambiguous result. The disadvantage is that in attempting to isolate from a stream or soil sample, even with a selective medium, there can be an abundance of other *Phytophthora-Pythium* species that may out compete and/or interfere with the growth of *P. ramorum*. It is also possible that *P. ramorum* could be dormant or nonviable.

P.lat multiplex PCR was robust, with no ambiguity in streams outside of the known infested area, or known positive streams. However, it would be less reliable in areas of *P. lateralis* infestation.

The nested protocol's round one results were consistent with the other tests. Round two results however were problematic. Many of the additional positives were consistent with stream history and so can be attributed to the added sensitivity of the second round amplification. Some of the additional positives were apparent false positives, coming from streams well outside the known *P. ramorum* infestation, that have not been found positive with any other assay.

The CSL assay results closely matched the results of the other tests, although on several occasions it gave false negatives. Adhering to the suggested strict cycle threshold it failed to detect *P. ramorum* in infested streams with a low inoculum load (WA46 and WA65).

While a *P. ramorum* culture is the most trusted positive result, it is clearly not the most sensitive assay. PCR assays have the advantage of being able to detect *P. ramorum* even if it is no longer viable. On the other hand, with the continuing discovery of new *Phytophthoras* it is difficult to know if *P. ramorum* specific primers truly are species specific.

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Stream Monitoring for Detection of *Phytophthora ramorum* in Oregon[1]

W. Sutton,[2] E.M. Hansen,[2] P. Reeser,[2] and A. Kanaskie[3]

Abstract

Stream monitoring using leaf baits for early detection of *P. ramorum* is an important part of the Oregon sudden oak death program. About 50 streams in and near the Oregon quarantine area in the southwest corner of the state are currently monitored. Rhododendron and tanoak leaf baits in mesh bags are exchanged every two weeks throughout the year. Leaves are sent to Corvallis for isolation and PCR diagnosis using the Winton and Hansen ITS primers. *P. ramorum* is regularly recovered from streams draining infested sites 5 years after eradication treatment. Because there has been a very good correspondence between culture results and PCR results from these known infested streams, we have changed to PCR only diagnosis on streams outside the known diseased area. If a PCR positive result is obtained, these baits and subsequent baits from that stream are cultured as well as assayed by PCR. In three watersheds *Phytophthora ramorum* was first detected using stream baiting. Follow up intensive ground surveys located infected tanoaks or other host plants. *Phytophthora ramorum* has been cultured from one additional stream but a source of inoculum has not yet been found.

Key words: *Phytophthora ramorum*, sudden oak death, PCR, stream monitoring.

Methods and Results

Rhododendron and tanoak leaf baits in mesh bags are floated in streams and exchanged every two weeks throughout the year. Leaves are sent to Oregon State University (OSU) for diagnostic testing. When stream monitoring first began in Oregon all bait samples were cultured using CARP+BH, a selective medium and PCR assay using the Winton and Hansen multiplex protocol. Over time confidence grew in the PCR assay so that currently initial testing of streams baits uses PCR only. If a PCR positive result is obtained from a stream not known to be infected, these baits and subsequent baits from that stream are culture and PCR assayed.

Culture vs PCR

Four years of data were analyzed comparing the two diagnostic methods (table 1). With PCR assay there are only two outcomes, positive and negative. PCR reliably detects *P. ramorum* in baits, even when it cannot be isolated in pure culture. When culturing however, there is a third outcome, another *Phytophthora* species.

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Phytophthora species are abundant in forest streams, and may mask or prevent the growth of *P. ramorum*. While the system is not perfect, 89 percent of the time the outcomes agree, three percent are mismatched and eight percent are suspected to be culture suppression due to other *Phytophthora* or *Pythium* species, or *P. ramorum* no longer being viable.

Table 1—Correspondence between culture and PCR results for detection of *P. ramorum* in 1,148 stream bait samples collected from 2003 to 2006

Diagnosis		
Culture	*PCR*	*Percent*
P. ramorum	Positive	6
No Ph sp	Negative	25
P. ramorum	Negative	1
No Ph sp	Positive	2
Other Ph sp	Positive	8
Other Ph sp	negative	58

Seasonality

Phytophthora ramorum is detected in streams by culturing and diagnostic PCR of leaf baits in all seasons of the year (table 2).

Eradication Sites

Phytophthora ramorum is recovered regularly from streams draining infested sites 5 years after eradication treatment (table 3). Detection rate has declined from 70 percent of sample intervals in 2003 to 39 percent of intervals in 2006.

Table 2—Number and percentage of monthly intervals in which *P. ramorum* was detected by culture or PCR in five streams draining eradication sites in a four year period (2003-2006)

	Winter		Spring		Summer		Fall	
Culture	8/31	26%	10/34	29%	9/35	26%	15/31	48%
PCR	18/52	35%	37/60	62%	41/60	68%	29/50	58%

Table 3—Frequency of detection of *P. ramorum* in 5 streams draining SOD eradication sites

	2003		2006	
Stream	# sample intervals	*P. ramorum* positive intervals	# sample intervals	*P. ramorum* positive intervals
WA1	17	71%	19	53%
WA4	24	50%	22	32%
WA6	15	33%	23	13%
WA9	23	87%	24	46%
WA12	24	96%	24	54%
Total	103	70%	112	39%

Early Detection Tool

Tentative belief that stream monitoring could be used as an early detection tool for *P. ramorum* has been proven well-founded. *Phytophthora ramorum* was detected using stream baiting in several watersheds before any infected plants were found. Three streams illustrate the utility of stream baiting for early detection of *P. ramorum* in tanoak forests .

Stream WA46 drains tanoak forest, flowing into the North Fork Chetco river from the east. Baiting began in March 2004, and the first PCR positive results were obtained in June 2004. Repeated ground searches revealed nothing, until November 2005 when three infected tanoaks were found (table 4). The site received eradication treatments that winter.

Stream WA65 drains an old-growth redwood-tanoak-Douglas-fir stand on state and federal land. It was first baited for *P. ramorum* in May 2004, and was first culture and PCR positive in October 2005. Despite earlier searches, infected plants were not located until April 2006, and the site received eradication treatments that year (table 5).

WA 31 is 2 km north of the quarantine area. It has been baited since March 2004. It was first PCR positive in August 2004, but was then negative until August 2005, when *P. ramorum* was cultured from a leaf floating in the stream. The next five sample times were PCR positive (*table 6*). Repeated ground surveys had failed to find the source of inoculum at the time of this meeting. On March 6, 2007 the Oregon Department of Forestry field crew located an infected tanoak 152.4 m (500 ft) up stream from this stream bait site.

Table 4—Monthly diagnostic results for stream WA46

	Mar04	Apr04	May04	Jun04	Jul04	Aug04	Sep04	Oct04	Nov04	Dec04
WA46	neg	neg	POS	POS	POS	neg	0	neg	neg	neg

Jan05	Feb05	Mar05	Apr05	May05	Jun05	Jul05	Aug05	Sep05	Oct05	Nov05	Dec05
neg	neg	neg	neg	neg	neg	neg	POS	neg	neg	neg	neg

Jan06	Feb06	Mar06	Apr06	May06	Jun06	Jul06	Aug06	Sep06	Oct06	Nov06	Dec06
neg	neg	neg	neg	0	POS	POS	neg	neg	POS	neg	neg

Table 5—Monthly diagnostic results for stream WA65

				May04	Jun04	Jul04	Aug04	Sep04	Oct04	Nov04	Dec04
WA65				neg	neg	neg	neg	0	0	neg	neg

Jan05	Feb05	Mar05	Apr05	May05	Jun05	Jul05	Aug05	Sep05	Oct05	Nov05	Dec05
neg	neg	neg	neg	neg	neg	neg	neg	neg	POS	neg	neg

Jan06	Feb06	Mar06	Apr06	May06	Jun06	Jul06	Aug06	Sep06	Oct06	Nov06	Dec06
neg	neg	neg	neg	POS	neg	neg	POS	neg	POS	neg	neg

Table 6—Monthly diagnostic results for stream WA31

	Mar04	Apr04	May04	Jun04	Jul04	Aug04	Sep04	Oct04	Nov04	Dec04
WA31	neg	neg	neg	neg	neg	POS	0	neg	neg	neg

Jan05	Feb05	Mar05	Apr05	May05	Jun05	Jul05	Aug05	Sep05	Oct05	Nov05	Dec05
neg	neg	neg	neg	neg	neg	neg	POS	POS	POS	POS	neg

Jan06	Feb06	Mar06	Apr06	May06	Jun06	Jul06	Aug06	Sep06	Oct06	Nov06	Dec06
neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

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Preservation of *Lithocarpus densiflorus* Diversity on California's Central Coast: A Cooperative Project With Area Residents[1]

Steven Swain,[2] Doug Schmidt,[3] and Matteo Garbelotto[3]

Abstract

The tanoak (*Lithocarpus densiflorus*) stands of California's central coast have suffered high mortality rates due to infection by *Phytophthora ramorum*. Treatment programs were put in place to test the efficacy of phosphonate compounds in real world situations, to preserve some germplasm and ecosystem elements for future research, and to help mitigate the buildup of standing fuels for local homeowners. Two approaches were involved, one using experimental plots distributed over a range from Marin County to Monterey County, and another involving a cooperative treatment arrangement with Big Sur residents. Preliminary results from the experimental plots suggest that tanoaks near the infection front, in spite of being asymptomatic, may not respond to treatment with phosphonate compounds. Our informal observations suggest that a cooperative treatment and research program with local area residents produces more community involvement and enthusiasm, and therefore higher treatment rates than informational approaches alone.

Key words: *Phytophthora ramorum*, treatment, phosphonate, efficacy, homeowner.

Introduction

In recent years tanoak (*Lithocarpus densiflorus*) stands in the Big Sur region of California's central coast have suffered extensive mortality due to the introduction of the pathogen *Phytophthora ramorum*. Aside from the risk of extirpation and the inevitable loss of genetic resources this poses, loss of significant numbers of tanoak may result in habitat degradation, particularly for those species dependent on tanoak acorns for survival. From a local homeowner perspective, the increased risk of fire from standing fuels is another cause for concern. In spite of extensive outreach efforts, it appears that most property owners do not attempt to treat their tanoaks until they show bleeding symptoms, at which point it is likely to be too late for treatment to be effective.

This project sets out to investigate the real-world efficacy of phosphonate treatments on tanoak, and to encourage local property owners to treat their trees prophylactically before symptoms are apparent, with the ultimate goal of preserving some tanoak diversity.

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Materials and Methods

The project consists of two treatment programs, both of which will require long-term post-treatment survival monitoring. All trees and/or plots in this study have confirmed infections within the immediate vicinity (within 200 m). None of the *Quercus* and/or *L. densiflorus* within the five main plot pairs were symptomatic at the start of the experiments, while only the very first symptoms of infection were visible on two remaining plot pairs (analyzed separately). Infection in the vicinity was confirmed first by plating symptomatic portions of *Umbellularia californica* leaves onto PARP selective media (Erwin and Ribeiro 1996), and if these tested negative, re-testing the leaves using CSL taqman molecular methods (Hughes and others 2006). The uninfected *Quercus* or *L. densiflorus* trees were treated with a phosphonate solution (Agri-Fos™, Agrichem Manufacturing Industries, Queensland, Australia) mixed with an organo-silicate surfactant (Pentrabark™, Agrichem Manufacturing Industries, Queensland, Australia) allowing penetration of the material into trunk tissues without drilling. The solution was applied with a commercially available model 425 Solo hand-pump backpack sprayer (Solo™, Newport News, VA, USA) at low to moderate pressures to a height of approximately 3 m until runoff began to occur.

Treatment Plots

The first program consists of clusters of one to five paired 20x20 m treatment and control plots (table 1). These were established by June 2006 and cover a range from Marin County in the north to Monterey's Big Sur coast in the south. Comparisons are to be made between treatment and control plots to assess treatment effectiveness under comparatively undisturbed real-world conditions. Additionally, two paired 20x20 m treatment and control plots were established at the same time in the Carmel Highlands under similar circumstances, except that the tanoaks in these plots had just begun to show the first symptoms of infection at the time of treatment (table 1).

Cooperative Treatments

The second treatment program is a cooperative agreement with private property owners in the Big Sur and Carmel Valley regions (table 1). Participants in the program have individual, high risk trees which receive two phosphonate treatments within the first year at no cost. Property owners are then responsible for applying the subsequent three treatments over five years. The treatments are uncontrolled.

Table 1—Phosphonate treatment sites

		Plot pair distribution and tally	
County	*Sites*	*# of plot pairs (treatment & control)*	*Total # of trees*
Monterey	4	5 (plus 2 symptomatic pairs)	176
Marin	1	5	249
Santa Cruz	1	2	100
Monterey	13	homeowners treatments (uncontrolled)	107

Results

While data is still being collected on the trees within the treatment plots and involved in the cooperative agreements, some trends have already become apparent.

Treatment Plots

Within one year of treatment, all *L. densiflorus* trees within the two "symptomatic" plot pairs in Carmel Valley were strongly symptomatic of disease, regardless of whether they were in treatment or control plots, or whether or not they were symptomatic at the time of treatment.

Cooperative Treatments

We visited more than 20 properties to test for presence of the disease, and 13 of them, collectively containing more than 100 trees, both tested positive for the presence of the pathogen and contained asymptomatic trees that had not yet been treated with phosphonate.

Discussion

Treatment Plots

The high levels of infection in the Carmel Valley plots which contained only a few mildly symptomatic trees at the time of plot establishment suggests that asymptomatic tanoaks close to the geographic disease front may in fact be seriously infected, and therefore cannot be expected to respond to treatment with phosphonate compounds. Treatment recommendations should be re-evaluated to insure that there is no expectation that asymptomatic tanoaks will respond if treated with phosphonate compounds when growing among infected trees. In the future, survival data from these trees will provide us with real-world treatment effectiveness on symptom-free stands when treatment was applied.

Cooperative Treatments

We used a "hands-on" approach, designed to increase public interest and participation in the treatment process while simultaneously striving to reach an ecologically important goal. To the best of our knowledge, prior to this program only a few property owners had prophylactically treated their tanoaks with phosphonate, totaling perhaps a few score of trees. This situation persisted despite educational outreach efforts and high tanoak mortality in the area. By engaging in a cooperative research and treatment venture with local property owners, this program appears to have increased the number of individuals prophylactically treating their trees by several fold, and we estimate that it at least doubled the number of treated trees over a one year period. If successful, this will have the effect of preserving some local germplasm and acorn production for future work, and may help to stabilize some of the ecological changes that may affect the Big Sur region. While we are not social scientists and subjective qualities like "public interest" are difficult to quantify, it does not mean that these aspects of treatment are not significant from an epidemiological perspective. There is an opportunity for further research here.

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Survival of *Phytophthora ramorum* Chlamydospores at High and Low Temperatures[1]

Paul W. Tooley and Marsha Browning[2]

Abstract

Chlamydospores were produced as described by Colburn and Shishkoff (Phytopathology 96:S25). Samples (5cc) of chlamydospores in sand inoculum were placed in 15 ml conical plastic test tubes and incubated at selected temperatures for 1, 2, 3, 4, and 7 days. Following incubation, tube contents were resuspended in 0.2 percent water agar and 1 ml was plated onto PARPH selective medium amended with 4 percent clarified V8 juice. Numbers of colonies resulting from germinated chlamydospores were assessed microscopically. High temperature treatments included 30, 35, and 40°C while low temperature treatments included 0, -10, and -20°C. All experiments also included chlamydospores placed at 20°C as positive controls. Near 100 percent survival was observed at temperatures of 0°C and for the 20°C controls for up to 7 days in the low temperature treatments, while almost no survival occurred at -10 or -20°C over the 7 day period. For the high temperature treatments, high levels of chlamydospore germination were observed over the 7 day period at 30°C and for the 20°C controls, while no growth was observed at 40°C. At 35°C, high levels of chlamydospore germination were observed at day 1, but growth declined steadily and was zero by 7 days. These results help define the temperature conditions under which chlamydospores of *P. ramorum* survive, and provide information to help define treatments aimed at inactivating chlamydospores in soil substrates.

Key words: *Phytophthora ramorum*, chlamydospore survival.

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Preliminary Observations of Heat Treatment to Control *Phytophthora ramorum* in Infected Wood Species: An Extended Abstract[1]

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Abstract

Identification of appropriate phytosanitary treatments that can be used for certifying solid wood packing material movement from areas infested or threatened by actionable plant pests and pathogens into uninfested areas is important. Heat treatment has been used on commodities to control fungal diseases and insect infestations for many years. The restricted use of methyl bromide has revived the interest on heat disinfestation for commodities. The purpose of this research is to determine the efficacy of heat treatment to control *Phytophthora ramorum* in wood species. Artificially inoculated and naturally *P. ramorum*-infected tanoak rounds and boards were heat-treated in a dry kiln set at the temperatures 50, 56, 60, and 65°C for 30, 45, and 60 minute durations. Core temperature, heating time, wet bulb, and dry bulb temperatures were monitored. *Phytophthora. ramoru*m was detected in wood using baiting and plating techniques before and after the treatment. Preliminary data showed that the required phytosanitary temperature of 56°C for 30 minutes might not be adequate to kill *P. ramorum* in wood. However, laboratory techniques have been redefined for subsequent tests using the temperatures of 50, 56, 62, and 68°C for 15, 30, and 60 minute durations.

Key words: *Phytophthora ramorum*, sudden oak death, heat treatment, tanoak.

Introduction

The potential to spread plant diseases by moving infected plants and plant materials from one location to another is well documented (Erwin and Ribeiro 1996, Davidson and others 2003, Shelly and others2005). Although kiln-dried lumber would likely experience temperatures lethal to *P. ramorum*, wood that is not kiln-dried could carry the organism. Current regulations for *P. ramorum* require the wood of hosts to be certified as debarked before they may be moved interstate. Research has suggested that *P. ramorum* could survive the recommended control measures such as removing the bark and air drying thus not completely mitigating the risk. There is a need for better understanding of the current recommended *P. ramorum* treatment methods as well as the identification of treatment alternatives for logs, bark, wood, and fire wood that are efficacious for *P. ramorum*. The restricted use of methyl bromide has revived the interest on heat disinfestation at 56˚C for 30 minutes for commodities. The

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purpose of this research is to determine the efficacy of heat treatment to control *P. ramorum* in infected wood that is not intended to be kiln-dried.

Objectives

This study was designed to determine the relationship between oven temperature, wood temperature, and *P. ramorum* survivability in infected wood.

Materials and Methods

Tanoak (*Lithocarpus densiflorus*), a hardwood host for *P. ramorum* with known commercial potential, was chosen as the specimen material for this project (Shelly and others 2006a, 2006b). The specimens were collected from the California Department of Forestry's (CDF) Soquel Demonstration Forest in Soquel, California. Twenty one trees with symptoms of sudden oak death disease were identified during a survey of the forest. Samples from suspect trees were collected and sent for PCR analysis to the California Dept. of Food and Agriculture (CDFA) Diagnostic lab, Sacramento and cultured at the University of California at Berkeley (UCB), Forest Products Laboratory (tables 1, 2). The 15 trees that tested positive for *P. ramorum* (ranging in diameter from 12 to 30 inches = 30.48 to 76.20 cm)) were cut down and three to four; eight-inch (20.32 cm) thick rounds were cut from the *P. ramorum* infected zones of each tree. In addition an equal-sized sample of non infected tanoak trees were also sampled for lab inoculation. Half of the field sample rounds, and lab inoculated samples were debarked prior to testing, the other half kept the bark. Short lumber sized samples (1-inch x 8- inch x 12-inch = 2.54 x 20.32 x 30.48 cm) were also inoculated and heat treated. All inoculations were placed at three depths in each specimen (at the surface, 15 mm deep, 30 mm deep and 50 mm deep). The field samples (rounds) and inoculated wood samples were heated in an 8.2 m^3 laboratory dry kiln. Heating tests were conducted at four temperatures (50, 56, 60, 65°C) and three time durations (30, 45, and 60 minutes) keeping the relative humidity constant at about 20 percent.Temperature was measured at each depth and the time duration measurements included both length of time the chamber was at the stated temperature and the length of time the wood was at the stated temperature. After treatment, samples were removed from the *P. ramorum* infected areas of each round and cultured to determine if the treatment was effective in killing the organism.

Table 1—PCR results of artificially inoculated-tanoak rounds in the laboratory (rounds* were collected from a sudden oak death free area)

	PCR Analysis	
Lab ID Round	*Real Time*	*Nested*
1	**+**	
2	**+**	
3		**+**
4		**+**
5		**+**
6		**+**
7	**-**	
8	**-**	
9		**+**
10	**+**	

*Laboratory samples were inoculated with *P. ramorum* infested shavings placed at 15, 30, and 50 mm deep in wood.

Table 2—PCR and culture results of naturally infested tanoak rounds collected from the Soquel Demonstration Forest in Santa Cruz, CA[1]

	PCR Analysis		
Tree ID	*Real-Time*	*Nested*	*Culture*
601	+		+
602	-		
603	+		
604	-		
605	+		
606		+	
607	+		
608	+		+
609	+		
610	+		
611	-		
612		-	
613	+		
614		-	
615	+		
616			+
617			+
618			+
619			+
620	+		
621	-		

[1] All samples were analyzed (Nested and RT-PCR) and cultured prior to heat treatment in a 8.2 m^3 experimental dry kiln.

Results and Discussion

Results from preliminary data are inconclusive and show low detection of *P. ramorum* in controls, pre-heat treated, and post-heat treated samples. Positive *P. ramorum* was found in 4 of the 12 tests, but only in 16.6 percent of the samples (table 3). This low detection of the pathogen in samples might be attributed to the delay between collection and processing of the samples, an uneven distribution of *P. ramorum* in collected samples, and the recognized difficulty of proving the presence of active *P. ramorum*. The isolation of one active *P. ramorum* sample treated at 56°C for 30 minutes suggests that this required phytosanitary treatment might not be adequate to kill *P. ramorum* in wood. Subsequent tests will be conducted with artificially inoculated boards in the laboratory and bay laurel/pears/rhododendron leaves will be used to maximize the detection. The chlamydospore germination will also be monitored.

Table 3—Experimental heating conditions used in this study[1]

	Time, minutes		
Temperature, ˚C	*30*	*45*	*60*
50	**+**	**+**	**-**
56	**+**	**-**	**-**
60	**-**	**-**	**-**
65	**-**	**-**	**-**

[1]Temperatures listed are target temperatures and differ slightly from actual temperatures attained. Positive *P. ramorum* was determined by wood tissue culture and/or chlamidospore germination.Samples were two rounds with bark, two rounds with no bark, and two boards used in each temperature x duration combination. Control samples were positive for *P. ramorum.*

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Evaluation of Fungicides for Control of *Phytophthora ramorum*[1]

S. Wagner,[2] K. Kaminski,[2] and S. Werres[2]

Abstract

As part of the project European *Phytophthora ramorum* Pest Risk Analysis (RAPRA) a wide range of fungicides was tested for *in vitro* activity against mycelial growth and zoospore germination of *P. ramorum*. A preliminary set of experiments was performed to study the effect of nine common fungicides specific for *Phytophthora* spp. Dimethomorph, copper-octanoate, and the combination of mancozeb and fenamidone inhibited mycelium growth and zoospore germination of *P. ramorum* isolates *in vitro*. Mancozeb alone did not inhibit mycelial growth effectively. Propineb, azoxystrobin, propamacarb, and cyazofamid did not effect mycelial growth and zoospore germination. Five resistant isolates towards metalaxyl–M in the European population were detected. Further testing *in planta* is required before the actual efficacy of each treatment can be evaluated.

Key words: *Phytophthora ramorum,* fungicides, mycelial growth, zoospore germination, *in vitro* tests.

Materials and Methods

Fifteen isolates of *P. ramorum* from different host plants were examined, 12 originated from Europe (11 isolates mating type A1 and one isolate A2) and three isolates were obtained from the USA (all mating type A2). The strains were isolated from nursery plants and from plants of natural habitats, e.g. public gardens or forests. Nine fungicides with different mode of action and active ingredients were tested (see table 1). Each fungicide was tested with seven different concentrations of the active ingredient (a.i.), ranging from 0.001 to 1.000 µg a.i./mL by the agar plate method (15 mL / Petrie dish) for mycelium growth and by the use of a spectrophotometric test (200 µL / microtiter plate) for zoospore germination. The classification of effective and not effective fungicide follows after calculation of the hypothetical concentration of the registered minimal effective dose according to the manufacturer.

Results and Discussion

Dimethomorph, copper-octanoate, and the combination of mancozeb and fenamidone were the most effective chemical compounds against *P. ramorum* with a complete

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inhibition mycelium growth and zoospore germination at fungicide levels of 0.1 to 10 µg a.i. /ml (Table 1). In contrast azoxystrobin, cyazofamid and propamocarb showed very less effectiveness on mycelium growth and on the germination of the zoospores. Even concentrations of 100 µg a.i./ml or 1000 µg a.i./ml active ingredient, which is 100 times higher than recommended by the manufactures guides, couldn't reduce completely inhibit the mycelium growth or the zoospore germination (Table 1). For mancozeb less inhibition of mycelium growth was measured at the highest applied concentration. As expected for a contact fungicide there was a strong reduction of the zoospore germination. Similar was the situation for propineb with less inhibition of mycelium growth, whereas only six isolates showed a reduction in zoospore germination.

The investigations with metalaxyl-M showed that for mycelium growth five isolates were sensitive, four isolates were less sensitive and five isolates were resistant to this compound during mycelium growth (all of them were of European origin). For zoospore germination the same five isolates were tolerant and 10 isolates reacted sensitive. All *P. ramorum* isolates which were tolerant towards metalaxyl-M came from European nurseries.

Results from the *in vitro* tests lead to the assumption that several potentially valid options for chemical control of *P. ramorum* could be available. Dimethomorph, copper-octanoate, and the combination of mancozeb and fenamidone appear to be useful active ingredients against *P. ramorum*. The study also showed that in the European population first resistant isolates for metalaxyl–M can be found. Further testing *in planta* is required before the actual efficacy of each treatment can be evaluated.

Table 1—Effect of fungicides on mycelial growth and zoospore germination of *P. ramorum* isolates (µg active ingredient / mL)

Trade name	Active ingredient	Mode of action	Inhibition of mycelium growth (µg / ml)	Inhibition of zoospores germination (µg / ml)
Acrobat®	Dimethomorph	systemic	1.0	0.1 – 1.0
Antracol® WG	Propineb	contact	100 - 1000	1.0 - 100
Cueva®	Copper-octanoate	contact	10.0	10.0
Dithane Ultra® WP	Mancozeb	contact	> 1000	1.0
Fonganil Gold®	Metalaxyl-M	systemic	1.0 - > 1000	0.01 - 1000
Gemini®	Mancozeb + Fenamidone	contact / systemic	1.0	1.0
Ortiva®	Azoxystrobin	systemic	100 - >1000	100 - 1000
Previcur N®	Propamocarb	systemic	> 1000	> 1000
Ranman®	Cyazofamid	contact	1000	100 - 1000

Susceptibility of Selected Ornamental Plants to *Phytophthora ramorum*[1]

K. Kaminski,[2] S. Wagner,[2] and S. Werres[2]

Abstract

Within the European project "Risk Analysis for *Phytophthora ramorum*" (RAPRA), susceptibility of economically and ecologically important plants in Europe towards *P. ramorum* was tested via *in vitro* inoculation methods. In these studies different species and/or cultivars of *Buxus*, *Calluna*, *Erica*, *Hedera*, *Rhododendron*, and *Vaccinium* were tested using a detached leaf or twig assay. Zoospore suspensions of five isolates, three European and two North American, were produced for inoculation by immersion of detached leaves and twigs, some of which had been wounded prior to the treatment. After incubation in a wet chamber, development of necrosis was measured.

In these *in vitro* tests all *Buxus* spp., *Hedera helix*, *Vaccinium corymbosum* and *V. macrocarpon* were classified as not susceptible. *Rhododendron simsii* and *Erica x darleyensis* developed only small necroses and were classified as slightly susceptible, the susceptibility of *R. simsii* varying with the cultivar. The cultivar 'Desta' was the most susceptible of the four cultivars tested. *Erica cinerea* was classified as moderately susceptible and *E. gracilis*, *E. carnea*, *Calluna vulgaris*, *Vaccinium myrtillus* and *V. oxycoccus* as highly susceptible. The cultivar 'Marleen' of *C. vulgaris* was significantly less susceptible than the three other cultivars.

Whether or not the plants had been wounded before inoculation was often irrelevant, though wounding did lead to larger necroses for some of the plant species, e. g. *R. simsii*, *V. oxycoccus*, and *V. corymbosum*. The aggressiveness of the isolates varied with the plant species tested.

Key words: *Phytophthora ramorum,* sudden oak death, ramorum blight, host range, *Buxus*, *Calluna*, *Erica*, *Hedera*, *Rhododendron simsii*, *Vaccinium*.

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Studies of Tissue Colonization in *Rhododendron* by *Phytophthora ramorum*[1]

Marko Riedel,[2] Stefan Wagner,[2] Monika Götz,[2] Lassaad Belbahri,[3] Francois Lefort,[3] and Sabine Werres[2]

Abstract

The knowledge on latency is of great importance to prevent the spread of *Phytophthora ramorum* with healthy looking plant material. To learn more about the tissue colonisation in *Rhododendron*, histological studies with epifluorescence microscopy have been started. Epifluorescence images showing *P. ramorum* structures in different tissues are presented. A first idea of *P. ramorum* development in Rhododendron root, twig and leaf tissue is offered for discussion. To improve and simplify the specific detection of *P. ramorum* structures under controlled conditions a method for the genetic transformation of *P. ramorum* with the reporter gene for Green Fluorescent Protein (GFP) was developed. With protoplast transformation transgenic *P. ramorum* strains could be produced for the first time. Integration of the transferred genes was proved by RT-PCR. Expression of the marker gene nptII could be verified by growth of transformed strains on medium containing antibiotics (geneticin). Expression of the GFP gene in hyphae, zoosporangia, germinating cysts and clamydospores could be demonstrated with a confocal laser scanning microscope (CLSM).

Key words: *Phytophthora ramorum*, CLSM, fluorescence microscopy, GFP, histology, genetic transformation.

Introduction

The proceeding worldwide spread and the expanding host spectrum of *P. ramorum* has become a serious threat to natural plant communities and commercial nurseries. To counter this threat, detailed knowledge about infection pathways and tissue colonization is essential. Infection of aerial plant parts is known to occur via splash water. Contaminated potting medium appears also to be a source for infection (Lewis and Parke 2006). But only limited knowledge is available about the details of infection of subterraneous plant parts like roots and stem bases. To investigate this, infection tests with container *Rhododendron* plants were performed and inoculated tissue was analyzed microscopically.

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Material and Methods

Inoculation and Histological Analysis

The European *P. ramorum* isolate BBA 9/95 was cultivated on carrot piece agar (Pogoda and Werres 2004) at 20°C with 16 h light for 14 days. Zoospores were released when water-flooded (7 ml distilled, sterile) plates were incubated 1 h at 4°C and 1 h at 20°C. Potted well rooted cuttings of *Rhododendron* `Cunninghams White´ were inoculated by application of 5 ml zoospore suspension (30,000 zoospores/ml) onto the surface of fresh irrigated potting soil. The inoculated plants were grown in quarantine chamber at 20°C with 16 h light. Samples of healthy looking plants were taken 8 days after inoculation. Other plants were sampled when disease symptoms occurred. Plant tissue was fixed in formaline-aceto-alcohol (FAA) for at least 24 hours. Handcuttings of stems, twigs, leaves and roots as well as whole roots were examined with light and epifluorescence microscopy.

Genetic Transformation

The protocol for the PEG mediated transformation of protoplasts (Judelson and others 1991) was slightly modified for transformation of *P. ramorum*. As a transformation vector the GFP-expression vector p34GFN (Si-Ammour and others 2003) and vectors based on Gateway™ technology were used in different trials. The transformed protoplasts germinated after 24 h incubation at 18°C in the dark and then were propagated on V8 agar containing 20 µg/ml of geneticin antibiotic (G418, Life Technologies).

Results and Discussion

Histological Analysis

Epifluorescence microscopy of infected plant tissue offers an excellent possibility to analyze the infection process without laborious pretreatments like clearing and staining. A fixation of samples with FAA enhances the autofluorescence of plant tissue and *P. ramorum* structures. Thus tissue type, tissue condition and pathogen structures can be indicated only by the color of autofluorescence. *Phytophthora ramorum* structures can be found in nearly all tissue of infected *Rhododendron* plants. In infected stems and roots with clear visible symptoms (discoloration) hyphae of *P.ramorum* are mainly localized in cortex and pith. In healthy looking stem and root segments located next to the discolored areas hyphae were found most often in the secondary xylem. In all stages of the infection process hyphae can be found in the tissue.

With one exception clamydospores were only located in discolored parts of infected *Rhododendron* plants. In discolored stems and leaves most of the clamydospores were localized in the cortex (stem) and palisade mesophyll (leaf). Less frequently clamydospores can be observed in the pith of necrotic stem segments. In stems, twigs and leaves the development of clamydospores can normally only be observed when host tissue is severely damaged which is indicated by severe discoloration and collapsing cell structures. In contrast clamydospores could be located in epidermis cells of healthy looking roots approx. two weeks after inoculation.

Production of zoosporangia on leaf surfaces could be documented using the vital dye FUN®1 (Molecular Probes). Development of zoosporangia was induced when infected leaves were moistened and kept at low temperatures (7°C). Sporangia developed on branching hyphae which were growing out of stomata on discolored leaf surfaces.

Genetic Transformation

With protoplast transformation transgenic *P. ramorum* strains could be produced for the first time. Integration of the transferred genes was proved by RT-PCR. Expression of the marker gene nptII could be verified by growth of transformed strains on medium containing antibiotics (geneticin). Expression of GFP gene in hyphae, zoosporangia, germinating cysts and clamydospores could be demonstrated with a confocal laser scanning microscope (CLSM). But in all of the transformed *P. ramorum* strains the expression of GFP appears to be low. For a practical use for instance in infection assays a stronger GFP expression is essential. In transgenic organisms the level of transgene expression is often influenced by the integration event of the transferred plasmids. Another important factor influencing the ability to express transgenes is the genotype of the recipient organism. The production of more transformations would provide a good chance to find an isolate with a higher expression of the transgenes. A second option focuses on the genotype of the recipient isolates. The *P. ramorum* wild type isolates used for transformation in the current project might not represent the ideal genotype for genetic transformation. A screening of other isolates could be successful to find the desired isolate.

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The Big Sur Ecoregion Sudden Oak Death Adaptive Management Project: Ecological Monitoring[1]

Allison C. Wickland,[2] Kerri M. Frangioso,[2] David M. Rizzo,[2] and Ross K. Meentemeyer[3]

Abstract

The Big Sur area is one of the most ecologically diverse regions in California. Land preservation efforts are well established in Big Sur, including numerous preserves, state parks and the Los Padres National Forest. However, there are still many conservation threats that cut across these areas including exotic species (plants, animals, and pathogens) and alterations of key natural processes such as a fire. Big Sur provides an ideal environment to address questions on the ecological consequences of *Phytophthora ramorum* due to the extensiveness of the forests, the relatively high impact of the disease in this area and the diversity of environments and disturbance histories. The Big Sur Ecoregion sudden oak death adaptive management project is a collaborative effort that brings together researchers (University of California Davis, UC Berkeley, UC Santa Barbara, University of North Carolina-Charlotte, United States Department of Agriculture - Forest Service) and land managers (Big Sur Land Trust, Santa Lucia Conservancy, Los Padres National Forest, UC Reserve System, Monterey Peninsula Regional Parks, California State Parks, private land owners). The main objectives of this project are to 1) establish a network of ecological monitoring plots; 2) evaluate impacts of sudden oak death (SOD) on ecosystem composition and dynamics; 3) develop a SOD management plan that compliments other landscape management goals; 4) test the efficacy of management actions; and 5) develop an outreach program that involves local communities.

To fulfill objectives 1 and 2, we are establishing a network of long-term ecological monitoring plots across the Big Sur Ecoregion. High-resolution, digital aerial photography integrated into a geographic information system (GIS) were used to map habitat types and tree mortality associated with *P. ramorum* in the Big Sur region. This information was the basis for a model built to randomly generate the location of the ecological monitoring plots stratified by forest type, level of tree mortality, fire history and land ownership (public versus private). In the plots, comprehensive data on the environment, vegetation, forest structure, disease level and site history is being collected. The location of each plot is spatially referenced and will be entered into a GIS database for analysis with other variables such as fire frequency, land use history and climate. Plots will be re-sampled over multiple years to monitor change.

Of the 300 proposed plots, 175 were established in the 2006 field season. Of these 175 plots, 99 contained trees that tested culture positive for *P. ramorum*. A preliminary look at the results shows that numerous plots have 100 percent infection of tanoak (*Lithocarpus densiflorus*) and sometimes coast live oak (*Quercus agrifolia*). In the *P. ramorum* positive plots, 72 percent of living tanoaks, 11 percent of living coast live oaks and 3 percent of living Shreve's oaks (*Quercus parvula* var. *shrevei*) were symptomatic. Standing stem mortality in

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positive plots was 23 percent for tanoak, 25 percent for coast live oak and 27 percent for Shreve's oak. However, standing mortality can be a misleading metric as many of the trees have fallen over and even decomposed in older infection sites. The high volume of large (>20cm in diameter) downed coarse woody debris found in positive plots as compared to negative plots (2.6 times the volume of tanoak and 3.4 times the volume of coast live oak in positive plots) supports this. A preliminary look at the data also suggests that variable levels of mortality are occurring in different stem size classes. For example, the standing mortality for tanoak stems in the larger stem size classes (>20cm) is more than 3 times that of the smaller stem size classes (1 to 5cm).

All 300 plots will be established by the end of the 2007 field season and the data collected will be used to a) evaluate the current distribution and spread of *P. ramorum* across the Big Sur region and b) provide baseline data on forest community composition and dynamics. Understanding the current spatial distribution of *P. ramorum* on the landscape, how this distribution is changing, and the underlying influences on establishment and spread of *P. ramorum* will be critical to making management decisions.

Key words: *Phytophthora ramorum*, sudden oak death, forest ecology, monitoring, Big Sur.

Investigating the Potential of Biological Control Against *Phytophthora ramorum*[1]

Timothy L. Widmer[2]

Abstract

Phytophthora ramorum is a unique organism in many ways, having a broad host range and both soilborne and aerial infection stages. This makes implementing effective control measures very complex. The use of biological control has been demonstrated against various *Phytophthora* spp. in general, but not specifically against *P. ramorum*. One aspect of biological control considered for use in this study was the use of microorganisms. Over 100 fungi, bacteria, and actinomycetes were isolated from natural field soil, leaf surfaces, and plant parts (endophytes). Some soil organism and endophyte isolates demonstrated strong growth inhibition towards *P. ramorum* colonies on agar plates. Encysted zoospores were completely inhibited from germinating on agar plates when pipetted 2 mm from active colonies of Sar-endo3, Alleg-endo2, Act-2Db, Act-3E, Act-3B1, and Act-3B2. Isolate Ch-1F inhibited zoospore germination 41 percent, while isolate CW-endo2 increased germination 27 percent compared to the control. Further tests using detached leaves, treated with some of these inhibitory organisms prior to inoculation with *P. ramorum* zoospores, reduced infection and leaf necrosis. Control leaves were 100percent infected with an average of 35 percent necrosis, compared to leaves pretreated with isolates CW-endo2, Sar-endo3, Act-3E, Act-3B1, and Act-3B2 were 60 percent, 60 percent, 40 percent, 40 percent, and 40 percent infected, respectively, and 21 percent, 19 percent, 33 percent, 17 percent, and 17 percent necrosis, respectively. Several *Trichoderma* spp. isolates were mycoparasitic, attacking the sporangia and chlamydospores. Another aspect of biological control considered in this study was the use of natural plant products. Caffeic acid amended to V8 agar medium at concentrations of 1 or 3 g/L inhibited the germination of *P. ramorum* zoospores 98 percent and 100 percent, respectively. Direct germination of sporangia was inhibited 25 percent and 100 percent, respectively. Rice bran extract, which contains caffeic acid derivatives, inhibited leaf necrosis caused by *P. ramorum* zoospores when first applied to Rhododendron "Cunningham's White" leaves before inoculation. Although preliminary, these tests do demonstrate a potential for natural plant products and microorganisms to limit the infectivity and spread, if formulated properly.

Key words: *Phytophthora ramorum*, biological control.

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