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Proceedings of the Sudden Oak Death Fourth Science Symposium

June 15-18, 2009, Santa Cruz, California

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Proceedings of the Sudden Oak Death Fourth Science Symposium

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

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

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

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Sudden Oak Death: A Global Update

Detection and Eradication of *Phytophthora ramorum* from Oregon Forests, 2001-2008[1]

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

Abstract

Sudden oak death (SOD), caused by *Phytophthora ramorum*, was first discovered in Oregon forests by aerial survey 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. The Oregon SOD program now consists of the following elements: early detection; delimitation of infested areas; treatment; research and monitoring; and host reduction in areas of probable disease spread.

Early detection relies heavily on aerial surveys (fixed and rotary wing aircraft) to detect recently killed trees, followed by inspection and sampling of all trees spotted from the air. Ground-based surveys (scouting from vantage points or transect-based surveys) compliment the aerial surveys in that they allow detection of infected trees in early stages of symptom development that would not be visible from the air. Stream baiting with rhododendron (*Rhododendron* spp.) and tanoak (*Lithocarpus densiflorus*) leaves has been a useful early detection tool, and in seven watersheds has detected *P. ramorum* before infected plants were found in the watershed. Fifty-eight streams in and near the Oregon quarantine area in the southwest corner of the state are currently monitored for *P. ramorum*. The collective results from these surveys clearly suggest that *P. ramorum* does not occur in Oregon forests beyond the Curry County quarantine area.

Eradication treatments consist of cutting and burning all infected and nearby host plants as soon as possible after detection. During the first 2 years of the eradication effort, all host vegetation within 15 to 30 m of infected plants was destroyed. In subsequent years, this distance was increased to a minimum of 30 to 100 m, reflecting observations on spread of the pathogen. On private and U.S. Department of Agriculture, Forest Service (USDA FS) land, all tanoaks are injected with herbicide (imazapyr or glyphosate) prior to cutting in order to prevent stump sprouting following treatment. In recent years, tanoak also has been removed in areas of likely disease spread in order to disrupt spread of the pathogen across the landscape. We expect to expand this effort in 2009 and 2010 with funds from the American Recovery and Reinvestment Act of 2009.

During the first 4 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

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eradication. That trend ended in 2005 when the number of new infected trees and the area infested began increasing. Since then, delays in completing treatments and consecutive years of unusually wet spring and early summer weather have contributed to spread of the disease, forcing the expansion of the quarantine zone from 6734 ha to 41956 ha in 2008. In 2007 and 2008 we found approximately 60 new infested sites each year (fig. 1, fig. 2).

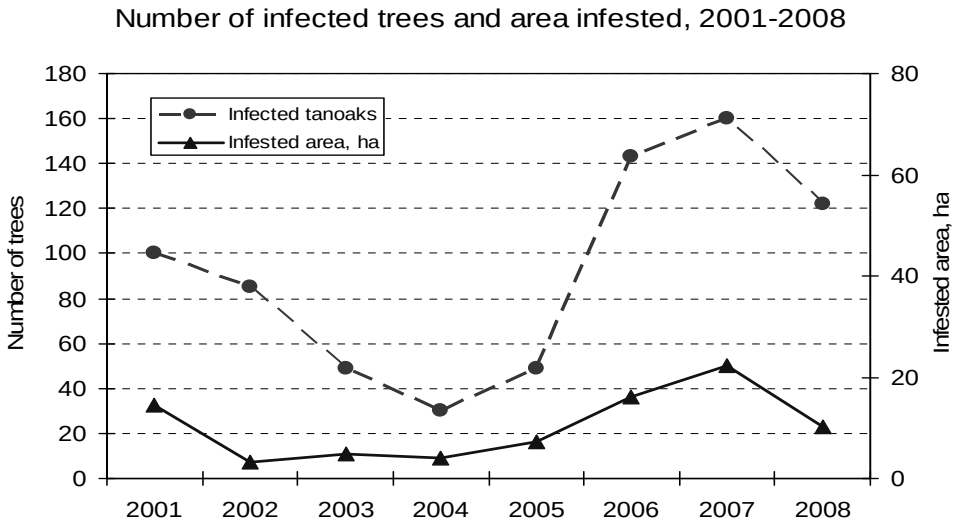

Figure 1—Sudden oak death trends in southern Curry County, Oregon, 2001 to 2008.

Between 2001 and the end of 2008 we completed eradication treatments on approximately 960 ha of forest at a cost of $4.3 million. Fund sources included the USDA FS (50 percent), State of Oregon (34 percent), U.S. Department of the Interior, Bureau of Land Management (USDI BLM) (10 percent), and U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS) (6 percent). There is no compensation to landowners for the value of timber or other resources lost as a result of the eradication treatments.

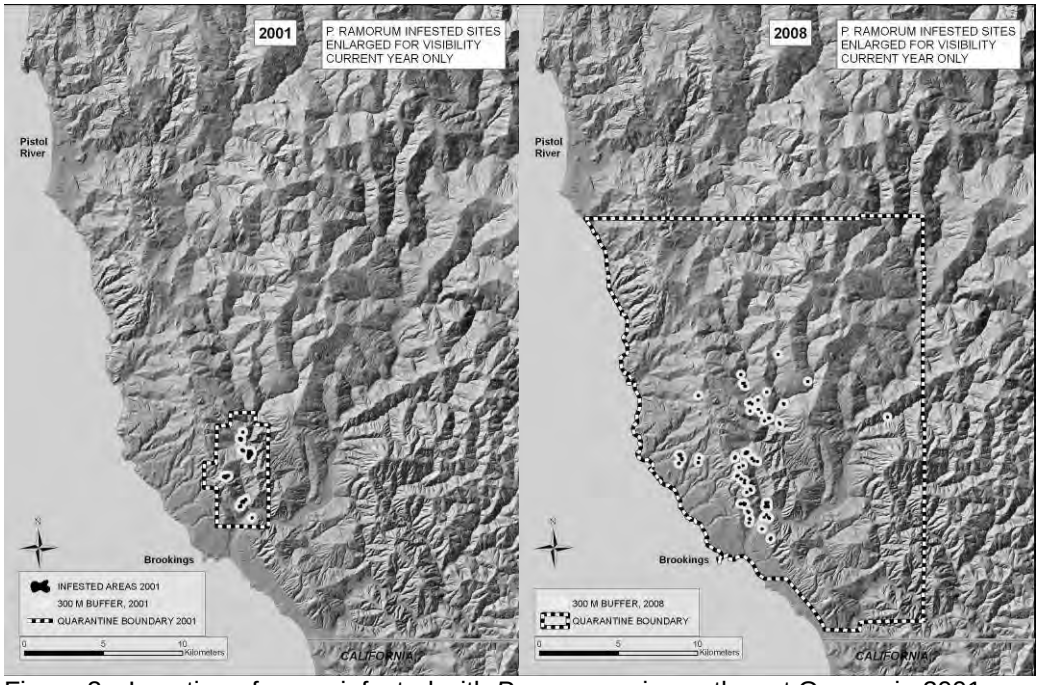

Figure 2—Location of areas infested with *P. ramorum* in southwest Oregon in 2001 and in 2008. Sites enlarged by a 300 m radius halo for visibility. White dashed line indicates quarantine area.

Research and monitoring during the past few years have yielded valuable information about the disease and its management in Oregon forests. Tanoak clearly is the most important host species in Oregon for two reasons; it is most susceptible and readily killed by the pathogen, and it appears to be essential for disease spread. We have found *P. ramorum* in forests only in association with infected tanoaks. Baiting rainfall collection buckets during various stages of the eradication process indicates that: 1) *P. ramorum* inoculum is available during rain events throughout the year; 2) inoculum is not generally distributed in the Curry County quarantine area, and; 3) inoculum is produced near infected trees before and during treatment, but not near the perimeter of the treatment areas or in apparently healthy stands.

Assaying soils and vegetation following eradication treatments has shown that *P. ramorum* is recoverable from soil on fewer than 50 percent of treated sites and is rarely found infecting plants. The lack of disease on treated sites suggests that the treatment is effective in most cases. Aerial application of Agri-Fos® is being tested as a means to disrupt disease spread, and results thus far indicate that aerially applied Agri-Fos® does have an affect on growth of the pathogen in treated trees.

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, thereby reducing damage to forests and minimizing the economic impact of quarantine regulations. Continued spread of *P. ramorum* despite the eradication effort is attributable in large part to latency of the pathogen in infected tanoak trees which allows pathogen spread between the time of initial infection and detection of symptoms. Administrative obstacles and inconsistent funding often extend the interval between detection of an infected tree and completion of eradication treatments, providing additional time for pathogen spread. These delays must be eliminated in order to improve effectiveness of the program.

Phytophthora ramorum and *Phytophthora kernoviae* in England and Wales - Public Consultation and New Programme[1]

Keith Walters,[2] Claire Sansford,[2] and David Slawson[2]

Abstract

Since the first reports in Great Britain (GB) of *Phytophthora ramorum* (2002) and *P. kernoviae* (2003), the death of a small number of infected trees and of heathland *Vaccinium* has been recorded. Initial policy against these pathogens was one of containment, with a view to eradication based on recommendations arising from Pest Risk Analyses (PRAs), whilst more evidence was gathered on their likely impact. Both pathogens have continued to spread slowly, mainly in the southern and western part of GB.

In 2008, a policy and science review, including a public consultation, was carried out in England and Wales, examining the current situation and options for future management of both pathogens. The consultation resulted in the conclusion that an increase in the current level of phytosanitary activity was required to reduce the potential risk of increased tree death and impacts to heathlands and heritage gardens in England and Wales.

A new 5 year programme of work aimed at reducing the risk of *P. ramorum* and *P. kernoviae* spreading further was launched by United Kingdom (U.K.) Ministers (March 2009); £4m was allocated for each of the first 3 years of the programme. Earlier research at woodland outbreak sites in southwest England showed that proactive clearance of sporulating hosts (whether infected or not), especially invasive *Rhododendron ponticum*, was effective in reducing pathogen inoculum and disease spread in woodlands, gardens and parks. This appears to have prevented further infection of trees at a number of woodland sites where clearance has been implemented. Consequently, future management in woodlands and the wider environment will include removal of infected and susceptible plants, and the identification and management of any new outbreaks. Activities will concentrate initially on high-risk, valuable sites, and vulnerable, ecologically important habitats.

Enhanced containment and eradication measures in infected gardens and nursery sites will also be undertaken, together with an education and awareness programme to build and disseminate best practice protocols, aimed at minimizing the risk that these pathogens pose.

A new research programme will improve our understanding of the two pathogens and the diseases they cause, enabling an update of the PRAs, thus informing programme activity, particularly with reference to disease control in a range of environments including heathland.

Introduction

Phytophthora ramorum and *P. kernoviae* are recently described plant pathogens that are considered to have been introduced separately to Europe and North America from

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an unknown origin or origins. *P. ramorum* was first confirmed in Great Britain (GB) in 2002, whilst *P. kernoviae* was first recorded in 2003. *P. ramorum* is present in 21 European countries (19 European Union (EU) Member States plus Norway and Switzerland). It is subject to emergency European Commission (EC) phytosanitary measures. Following an EU-wide Pest Risk Analysis (PRA) (Sansford and others 2009), the EC Standing Committee for Plant Health (SCPH) has provisionally agreed that *P. ramorum* should become listed as a harmful organism within the EC Plant Health Directive (2000/29/EC as amended), with phytosanitary measures yet to be defined and agreed. *P. kernoviae* is recorded in Great Britain (GB) and in Eire, as well as in New Zealand. A U.K. PRA for *P. kernoviae* (Sansford 2008) was also reviewed by the EC SCPH, and there has been provisional agreement that *P. kernoviae* should be subject to emergency EC phytosanitary measures, again yet to be defined and agreed.

In 2008, the Department for Environment, Food and Rural Affairs (Defra), the Forestry Commission, and the Welsh Assembly Government held a policy and science review on *P. ramorum* and *P. kernoviae*, including a public consultation. This examined the historic and current situation as well as the U.K. PRAs for both pathogens (Sansford and Woodhall 2007, Sansford 2008), and proposed options for management of these pathogens in the future. The public consultation offered three options: (i) continue to meet EC minimum requirements on the control of *P. ramorum* and remove all controls against *P. kernoviae* other than maintaining a ban on the movement of infected plants to other countries; (ii) undertake an enhanced programme of activity aimed at reducing the level of inoculum of both species to epidemiologically insignificant levels; (iii) continue containment/eradication activity with the objective of containing the spread whilst gathering further evidence prior to decisions on long-term action.

The third option was ruled out, principally because of the risk of the diseases moving into the exponential phase of the disease progress curve, thus increasing control costs and/or eliminating the potential of implementing option two. The majority of the responses to the consultation were in favour of option two or a variation of option two. The review and consultation recommended increasing current activity levels to reduce the risk of significant tree death and impact on heathlands, and identified the approaches to achieve this.

As a result of this review and consultation, and, its accompanying impact assessment and subsequent business case, a new Defra-funded "*Phytophthora ramorum* and *Phytophthora kernoviae* Disease Management Programme" was approved, to be delivered via Defra's Food and Environment Research Agency (Fera). This programme aims, amongst other objectives, to reduce pathogen inoculum to epidemiologically insignificant levels, by removing sporulating/potentially sporulating host plants from high-risk areas.

Risks (Great Britain)

In GB both *P. ramorum* and *P. kernoviae* are subject to an eradication/containment programme and so their full potential in terms of impact on the environment has not been realised. The west and southwest of GB seems particularly favourable to both pathogens in woodlands, heathlands and managed gardens.

In GB, *P. ramorum* and *P. kernoviae* cause leaf blights or dieback on a wide range of shrub hosts and some trees; these produce inoculum which can infect the stems of susceptible trees, causing bleeding bark cankers which can girdle and kill affected trees. Evergreen rhododendron (especially *Rhododendron ponticum*) is the main sporulating host that drives woodland epidemics, with beech (*Fagus sylvatica*) and some oak species (*Quercus* spp.) being particularly threatened by both pathogens. In GB, trees with bleeding bark cankers have, to date (summer 2009), all been in close proximity to infected rhododendron, particularly *Rhododendron ponticum*. Studies on the effect of *R. ponticum* clearance in infested woodlands, as part of recent research, has shown a reduction in inoculum levels and an apparent lack of infection of new trees within the study period. Recent surveys in GB woodlands have recorded sweet chestnuts (*Castanea sativa*) showing foliar symptoms (and stem cankers) caused by *P. ramorum*; as a sporulating host, this, as well as other species, can act as inoculum sources for infection of other trees. Ornamental plants and trees in heritage gardens can also be seriously affected, and those that are sporulating hosts will drive epidemics.

Heathland plants, especially *Vaccinium* species, are considered to be at high risk, having initially proven to be susceptible in laboratory experiments and subsequently becoming infected by both pathogens in GB. *V. ovatum* has been recorded as a natural host of *P. ramorum* in forests in North America, and in GB, *V. myrtillus* was recently found infected with *P. kernoviae* in woodland and heathland as well as with *P. ramorum* in heathland. *P. ramorum* has been found on nursery plants of *V. vitis-idaea* in GB and on *Calluna vulgaris* in Poland. The favourable climatic conditions in Cornwall have resulted in dramatic spread on sites with *V. myrtillus* and significant plant mortality. Both pathogens have the potential to cause further damage to GB heathland. For example, in Cornwall alone there is about 11 000 hectares of heathland within 10 km of currently infested sites.

Under GB conditions, both pathogens have the potential to produce spores all year round on rhododendron and possibly on other hosts which have not yet been studied. The clearance of sporulating hosts is likely to be the single most effective control measure to reduce disease spread in the wider environment (woodland, heathland, gardens, and parks), thus protecting vulnerable trees, shrubs, and heathland plants.

Outbreaks (England and Wales)

In England and Wales, between April 2002 and December 2008 there were 248 outbreaks of *P. ramorum* at 221 sites in locations other than nurseries, of which 84 have been eradicated (table 1). These woodland or garden/park sites are fairly widely distributed, but the highest incidence and severity of disease has been in the south and west of England and in south Wales (fig. 1). These areas of the country appear more favourable for the pathogen and for disease development, since they are mild and wet. Although the number of trees that are known to have developed bleeding cankers is low (approximately 28), it is increasing; a few trees have also developed bleeding cankers outside of the southwest region. Ornamental plants in historic gardens involved in tourism have been badly affected by the pathogen and some rare or historically important specimens or collections are now considered to be at risk. Visitors to some historic gardens have complained about the appearance of infected plants; gardens which rely on spring flowering rhododendrons and camellias to

attract visitors have been most affected. Nurseries have mainly been affected by the phytosanitary measures that have been implemented to try to prevent spread to the environment (see below). Between April 2002 and December 2008 in England and Wales there were 626 nursery outbreaks at 526 sites of which 541 have been eradicated (table 1).

Figure 1—Distribution of (non-nursery) outbreak sites for *P. ramorum* (pink dots) and *P. kernoviae* (blue dots) in England and Wales (2003 to 2008).

Between October 2003 and December 2008 there were 66 outbreaks of *P. kernoviae* in England and Wales on managed and unmanaged land, with an additional five outbreaks (at four sites) in nurseries and garden centres (table 1). All but five are subject to ongoing eradication or containment action.

Table 1—Outbreaks of *P. ramorum* and *P. kernoviae* in England and Wales between April 2002 and December 2008

Pathogen	Location	Total	Eradicated	Ongoing
Phytophthora ramorum	Nurseries and Garden Centres	626 (526 sites)	541	85
	Managed and Unmanaged Land	248 (221 sites)	84	164
Phytophthora kernoviae	Nurseries and Garden Centres	5 (4 sites)	4	1
	Managed and Unmanaged Land	66 (66 sites)	1	65

The number of new outbreaks recorded has been at a low annual frequency (and at similar levels between years) over this period (fig. 2). The most significant damage has again been in the southwest of England (Cornwall and one site in Devon) and at five sites in south Wales, with only one finding in a managed garden in northwest England. In September 2008, a site in Kent was found to be infested with *P. kernoviae* (this is thought to be an isolated site linked to plant movements from

Cornwall). Although the number of trees that have developed bleeding cankers is low (approximately 60), it is increasing, and a few trees with bleeding cankers have already died. As is the case for *P. ramorum*, ornamental plants and trees in managed gardens involved in tourism have been badly affected, and some rare or historically-important specimens continue to be at risk.

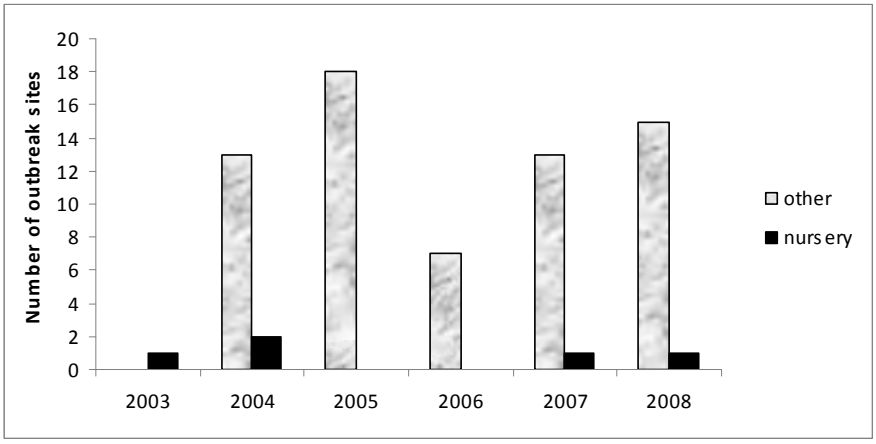

Figure 2—The number of outbreaks of *P. kernoviae* in nurseries and garden centres or other (managed and unmanaged land) sites in England and Wales in each year between 2003 and summer 2009.

The longer-term risk from both pathogens may increase if new isolates are introduced or if climatic conditions become even more favourable. If the pathogens are not controlled, it is not yet clear when or whether the whole of GB would become affected. However, in the absence of enhanced control measures, spread into and within the environment is predicted to increase. The scale of environmental damage is uncertain.

Movement of Infected Plants

The main means of long-distance spread in GB is thought to be by the movement of infected plants. However, there have been limited findings of *P. kernoviae* in the nursery trade, with the first GB findings being in woodland surrounding a nursery. Both pathogens are considered to be of exotic origin and introduced to GB on imported plants. Current EC measures for *P. ramorum* require that (a) official surveys be reported back to the EC at the end of the year and (b) import controls are imposed on all listed susceptible hosts imported from the U.S. (as well as on a separate list of species for susceptible wood), that there is a prohibition on imports of susceptible bark from the U.S. (same listed species as those for susceptible wood), and that there is internal movement controls in the EU (plant passporting) of *Rhododendron* (excluding *R. simsii*), *Viburnum*, and *Camellia* (the three most commonly affected traded genera in the EU), with statutory action to be taken on findings. These genera can only be moved from nurseries which have been officially inspected and found free from the disease, or where appropriate eradication measures have been taken. Consignments of these plants must be accompanied by plant passports (used already in the EU to manage risks from a number of other plant pests and pathogens). This aims to ensure that plants moved in trade are free of the pathogen and, if symptoms develop after movement, the infection can be traced back to the originating nursery and follow-up inspections carried out at sites which have

received plants from the same batch. There are no official EC measures in place for *P. kernoviae* but these are envisaged.

New *Phytophthora* Programme

The new Defra *Phytophthora* Programme will address the risks posed by the two pathogens through three main workstreams, namely: disease management and surveillance, awareness and behavioual change, and research.

The disease management and surveillance workstream will substantially increase the current level of phytosanitary activity, with the aim of clearing sporulating host plants (principally *R. ponticum*, but also other infected hosts) from known infested sites. The workstream may also involve removal of uninfected rhododendron or other potential sporulating hosts to protect vulnerable woodland and heathland, coupled with a national surveillance programme to monitor changes in disease distribution in England and Wales on a regular basis, and to identify and control new outbreaks. The scientific basis for this work will remain the comprehensive Pest Risk Analyses collating and interpreting published and unpublished research, but advantage will also be taken of the potential of modeling techniques developed recently in relation to *P. ramorum* outbreaks in California (Meentemeyer and others, these proceedings; Filipe and others, these proceedings; Vaclavik and others, these proceedings). Containment and eradication measures in infested parks and gardens will also be enhanced. An enhanced regime of checks and controls on commercial plant trade (nurseries) will be applied, and proactive management of high-risk sites will be encouraged.

The awareness and behavioural change workstream will primarily work with all stakeholder groups to develop and coordinate the drafting of joint codes of practice. These will improve biosecurity through better operational procedures both in management of established plants and through better buying and selling protocols. The lead for this work will be shared with stakeholders themselves to promote recognition of commercial as well as statutory plant health and biological requirements, and to facilitate uptake by industry. Initial work in this area has resulted in a suite of biosecurity protocols for heritage gardens developed by the National Trust (for England, Wales, and Northern Ireland) and representatives of the original Defra *Phytophthora* Programme (Wright and Slawson, these proceedings). A significant programme of work will also be undertaken to further raise awareness of the diseases amongst a broad range of stakeholder groups.

The science, policy, and operational needs of the disease management and surveillance, and the awareness and behavioural change workstreams will be supported by a research workstream. Research funding of up to £1.5 million is scheduled for the 5-year period of the Defra Programme, but to ensure that output is available to inform and guide the delivery of other elements of the wider programme, it will be front-loaded with annual funding of about £400,000 per year over the first 3 years of the programme. The research programme will aim to build upon previous work to refine further knowledge of pathogen and disease characteristics and spread in GB and develop tools for better containment and eradication in the future. In particular, there will be increased study into the impact of these pathogens on *Vaccinium* in the natural environment.

Measures of Success

If successful, the Defra *Phytophthora* Programme will result in an environment where trees and heathland are not generally threatened by *P. ramorum* or *P. kernoviae,* and where the commercial plant trade and historic and public gardens are applying codes of practice which reduce the risk of pathogen and disease spread through proactive biosecurity measures (which will benefit the control of these pathogens and others which are also present or may be introduced in the future). Thus, the social and environmental benefits that society enjoys from woodland and heathland, including sites of special scientific interest (SSSI) and visiting historic gardens, would be maintained.

At a high level, the disease management and surveillance workstream, if successful, would result in the protection of the wider environment from the risk of significant further tree and heathland infections. The eradication of long-lived spores (chlamydospores for *P. ramorum*) will be very difficult, as will eradication in heathland habitats, thus the aim is to control the risk of further new infections by reducing inoculum to levels which reduce the rate and potential for longer-distance spread to new sites. Success will be measured through national surveys of vulnerable sites and by measuring the percentage of new survey sites found to be infested. A reduction in the percentage of newly surveyed sites found to be infested would demonstrate a slowing of pathogen and disease spread.

The surveillance programme will aim to identify affected parks, gardens, woodlands, and heathlands through surveys, and to support the subsequent removal of all *R. ponticum* from these sites in England and Wales (currently estimated at approximately 450 ha) and potentially other known and potential sporulating hosts. The programme aims to complete the clearance of *R. ponticum* within the first 5 years of activity. Progress towards this total will be monitored and reported annually. A programme of surveillance and clearance of rhododendrons from uninfested high-risk sites which are valuable or ecologically important habitats will be developed.

With regard to the movement of *Phytophthora* in traded plants, the number of confirmations of infected material identified as a result of official inspections at nurseries and garden centres will be monitored, with the aim to achieve further reductions from the current 1 percent of material inspected. The results of recent monitoring has indicated that positive findings on nurseries have reduced from 3 percent to 1 percent of inspected material as a result of emergency action taken during the last 5 years.

Success of the awareness and behavioral change workstream will be measured by the degree to which the horticultural industry and historic and public gardens have developed suitable codes of practice which reflect improved biosecurity procedures and a responsible approach to disease management. The programme aims to co-ordinate activity across the sector to facilitate a joined-up approach. It is proposed that codes of practice should be in place within the first 3 years of the programme. In addition, the workstream aims to result in a better informed public about the pathogens/diseases, through an education and awareness campaign. This, combined with the industry codes of practice, will improve general disease awareness and management by the public.

A review of science to identify science gaps related to policy/management needs, priorities for a proactive research programme and strategy for commissioning new work will be completed during the first year of the research workstream. By year 3 of the programme it is intended that this will have contributed to refinement of our understanding of factors driving pathogen spread and disease development, and to a better understanding of risk to heathland environments; as well as comparative risks between *P. ramorum* and *P. kernoviae*. Improved disease management or control methods are intended to be established for heathland environments, together with improved approaches for socio-economic evaluations of the impacts of both pathogens and cost-benefit analyses of the measures.

Discussion

The principal objective of this programme is to support the development and maintenance of a healthy, resilient, productive, and diverse natural environment. Disease control under this programme is especially targeted to protect trees and native heathland from further infection. The main method of disease control is the removal of *R. ponticum*, a non-native invasive weed, which will also contribute to an increase in biodiversity by allowing native species to repopulate (as has been recorded in a test site in Cornwall). *P. kernoviae* has spread rapidly through *V. myrtillus* in heathland environments and is thought to have the potential to spread rapidly to other heathland sites (many of which are denoted as SSSIs). *P. ramorum* was more recently recorded on *V. myrtillus* in woodland in GB. The diseases will not only impact on the susceptible plant species, but also on the rare vertebrate and invertebrate species for which they provide a unique habitat. The U.K. has 20 percent of the world's lowland heath and approximately 75 percent of the total resource of upland heath. The U.K. government has a public service agreement target for 95 percent of the area of sites of SSSIs in England to be in 'good condition' by 2010. Much of the heathland resource is notified as SSSI and these habitats form a significant proportion of the total SSSI area. Disease control will help to maintain these SSSIs.

The impacts of these pathogens and the diseases they cause will principally be felt in rural areas (generally those associated with tourism). Infection in parks and gardens open to the public, and in heathland areas, could affect visitor numbers through the loss of plants that attract the public. In some areas of the country (particularly the southwest), local economies are highly dependent on the tourism generated by these gardens and landscapes.

The new *Phytophthora* programme will also increase the general awareness of plant disease and good biosecurity measures in the nursery and garden centre trade and of consumers. This will contribute to sustainable patterns of consumption and production and support the further development of a thriving farming and food sector, with an improving net environmental impact.

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Phytophthora Concerns

Some Challenges of Recognizing Invasive *Phytophthoras* and Finding Their Origins[1]

Everett Hansen[2]

Introduction

Discovering the origins of *Phytophthora ramorum* remains a challenge. To improve our chances of finding the origin of *P. ramorum* or any other introduced organism, we need to be sure of our motivation, because success will require persistence. We need to be able to distinguish indigenous from exotic organisms, to know what to look for and recognize when we have found it, and we need a practical "search image" to guide the discovery and sorting of candidate organisms.

Perhaps the most frequently asked question about *P. ramorum* and sudden oak death is "Where did it come from?" Despite several expeditions to far off lands (Goheen and others 2005) and repeated searches closer to home, the answer is unchanged: "I don't know. Maybe China." Our ignorance is frustrating, but shouldn't be surprising. We actually know the answer for very few pathogens. Perhaps it is timely to reexamine our approach. To improve our chances of finding the origin of *P. ramorum* or any other introduced organism, we need to deal with three additional questions: "How do we know it was introduced in the first place?" "Why do we need to know?" and "Where should we look and what should we be looking for?" The first is a necessary challenge to our epidemiological and genetic assumptions about invasive species of pathogenic fungi. The second is a question of motivation and commitment, and "Where to look?" is as much a question of opportunity as biology.

Exotic or Indiginous?

The question is not as trivial as it may sound. Sometimes the actual introduction of an organism or the beginning of an epidemic is noted, and there is sufficient documentation to be sure that it truly is a new phenomenon. More often, we can't be sure that the apparent novelty isn't an artifact of no one looking before.

In the absence of clear "trace-back" information on origins, we usually invoke two lines of evidence to separate native from introduced pathogens. We suppose that native pathogens have evolved with their host plants in a particular ecosystem, with checks and balances to prevent catastrophic epidemics. Certainly we don't expect that an indigenous pathogen would threaten the evolutionary reproductive potential of a population of a host species. We also suppose that populations of native pathogens will exhibit more genetic diversity than introduced organisms that likely exploded from one or a few individuals that were first established. In the case of

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heterothallic organisms, with two mating types, we expect to find both in the region of origin.

Phytophthora ramorum provides a relatively straightforward test of these assumptions. It appeared recently and suddenly, in a populated area with an abundance of plant pathologists, affecting an iconic and abundant tree (coast live oak, *Quercus agrifolia*) (Rizzo and others 2005). Sudden oak death has a clear beginning in both California and in Oregon, and it continues to spread (Hansen and others 2008) (table 1). Genetic markers allow us to track its further spread around the world and within North America (Goss and others 2009, Hansen and others 2003, Ivors and others 2004, Mascheretti and others 2008, Prospero and others 2009, Werres and others 2001). *P. ramorum* kills trees of all ages in expanding areas of mortality. Tanoaks (*Lithocarpus densiflorus*) are especially vulnerable, and their regional survival is threatened by this pathogen. *P. ramorum* did not coevolve with tanoak or coast live oak. Introduced organisms do not necessarily cause dramatic epidemics, however.

Table 1—Retrospective detection of dead tanoaks on color aerial photographs at five sites in Oregon where *P. ramorum* was confirmed as cause of death in 2001 (Hansen and others, 2008)

	Number of symptomatic tanoak trees visible on color aerial photos			
Site	**1996**	**1998**	**2000**	**2001**
10	0	1	7	110
11	0	0	4	31
17	0	0	1	12
18	0	0	1	14
33	0	0	1	32

Phytophthora ramorum is heterothallic (Werres and others 2001), but only one mating type (A2) is present in North American forests and only the opposite mating type (A1) is present in Europe (Ivors and others 2004). Neither population is sexually reproducing; the origin must be elsewhere. Presence of only one mating type is one kind of evidence for a genetic "bottleneck." In many cases, introduction must have involved only one or a few individuals, and the resulting epidemic population will have a correspondingly narrow genetic diversity, especially if it is limited to clonal reproduction, as is *P. ramorum* when only one mating type is present.

Measuring genetic diversity requires many isolates of a population, and demonstrating a "bottleneck" requires information on the diversity of the originating population as a basis for comparison. Although "limited genetic diversity" is often cited in support of a conclusion that a *Phytophthora* species is of exotic origin (Cooke and others 2005, Linzer and others 2009, Ivors and others 2004), this evidence is actually very difficult to evaluate. DNA fingerprinting using "AFLP" markers is often used, but differences in sample sizes, sources, and statistical treatments prevent comparisons between studies. A study of *P. quercina* in Europe for example, found all isolates to be closely related, but each was genetically unique. A similar study of *P. nemorosa* in California found that most isolates had identical DNA fingerprints. Both studies concluded that the organisms were introduced, but in neither case was an indigenous population used for comparison. The interpretation is

still more difficult because these examples are both homothallic organisms, and inbreeding would be expected to narrow genetic diversity, even in the area of origin.

Why Look?

"Why do we need to know where it came from?" is a question of motivation. Can we justify the expense and the mental and physical exertion necessary to undertake the search? It is fun to go fishing for *Phytophthora* in exotic places. It usually doesn't take much persuasion to get plant pathologists to sign up for such expeditions, but entertainment value doesn't appear on many grant proposals. Biosecurity is the most common official justification. But will we act on the information, strengthening quarantine regulations for example, or will global politics and economics trump risks to domestic agriculture and forestry yet again? There are other important uses for this information, however. Discovering origins is fundamental to basic studies of evolution and speciation. It is necessary for testing underlying ecological assumptions and theories of population genetics and coevolution. Again, *P. ramorum* illustrates these points.

The threat posed by *P. ramorum* to forests and woody plant horticulture around the world is clear. It is a known, named threat to global biosecurity, and quarantine regulations are in place to halt its movement to new areas. Knowledge of its lands of origin, however, will add only marginally to the effectiveness of these regulations. On the other hand, recent work on the population genetics of this dangerous pathogen (Goss and others, unpublished) suggests a complex evolutionary history, and studies of indigenous populations, once found, will be very revealing of the processes of isolation and speciation that lead to such complex pathogenic potential.

Phytophthora ramorum, as we know it today in North America and Europe, is comprised of three reproductively isolated clonal lineages, NA 1, NA 2, and EU 1 (Figure 1). Goss and colleagues concluded that:

- *P. ramorum* lineages are descended from a sexually reproducing population.
- The age of the three lineages is estimated to be between 1.5 and 5.4 million years.
- The lineages have probably been separated for about one tenth of their evolutionary history.

Thus the most recent split between lineages (NA2 from NA1) was a minimum of 165,000 years ago.

The ancestral, sexually reproducing population may not even exist anymore! We are probably looking for three separate origins for the three lineages.

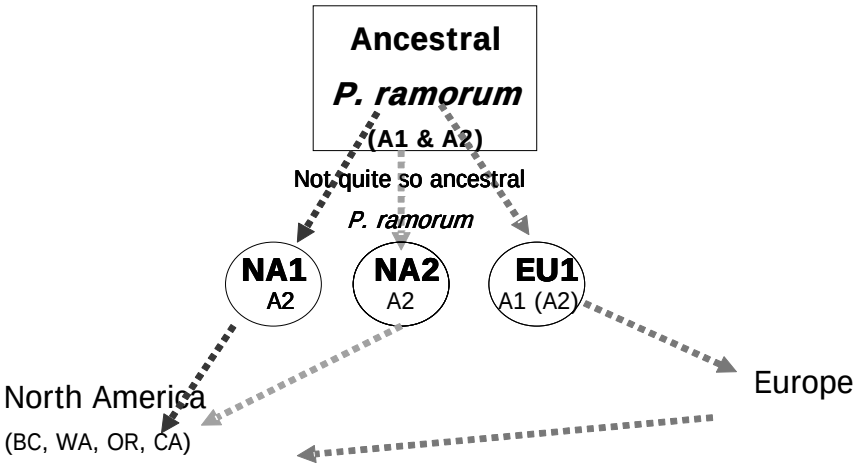

Figure 1—Hypothetical origins of *Phytophthora ramorum* as we understand it today.

Where to Look?

We usually jump right into the question "Where should we look for the source population of (for example) *P. ramorum*?" A typical analysis starts with a host - *P. ramorum* is virulent on rhododendron - and jumps into an expedition. So let's go look where rhododendrons come from (Goheen and others 2005). A better first step, however, may be to check existing culture collections and DNA sequence databases for unidentified or misidentified isolates. The conspecificity of *P. ramorum* in Europe and North America was first suspected based on morphological similarity between the new fungus from California, and an unidentified isolate in Germany. Similarly, *Phytophthora* isolates closely resembling *P. kernoviae* were found among old unidentified isolates in New Zealand, and new, lethal outbreaks of *Phytophthora* on alder in Australia and Foster City California were matched to the new species *P. siskiyouensis* through GenBank (Rooney-Latham and others 2008).

The so-called "alder *Phytophthora*" provides another example. *P. alni* was isolated from dying alders in Europe and shown to be cause of a dramatic new disease. It appeared to be a new, exotic, invasive pathogen based largely on its apparent sudden appearance and the destruction it caused. A search of the genetic databases showed it to be unique, but closely related to well-known organisms. Further genetic investigation demonstrated that *P. alni* is a hybrid species of complex parentage (Brasier and others 2004, Ioos and others 2006). Very recent surveys of remote streams and soils associated with wild alder stands in Alaska have yielded one of the putative parents (Adams and Trummer, this proceedings). The new isolates do not appear to be causing any dramatic disease in Alaska; this indeed may be at least one of the "origins" of the alder *Phytophthora*.

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Spread of *P. ramorum* from Nurseries into Waterways—Implications for Pathogen Establishment in New Areas[1]

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Abstract

In the United States, water and soil baiting have been part of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS) Confirmed Nursery Protocol (CNP) to prevent the spread of *Phytophthora ramorum* from infected nursery stock since 2005. Additionally, the U.S. Department of Agriculture, Forest Service (USDA FS) has, since 2006, supported the national early detection stream baiting survey of forests and urban areas to monitor the potential spread of this pathogen from nurseries and other high-risk settings. These surveys have been conducted in cooperation with universities and state forestry agencies. As a result of these activities, *P. ramorum* has been detected in waterways at 10 sites in six states between 2006 and June 2009 (fig. 1).

Below is a brief overview of these positive sites.

Florida - In 2006 and 2007, *P. ramorum* was recovered from standing and flowing water inside, but not outside, a retail garden center where *P. ramorum*-positive plants were present. In February 2008, *P. ramorum* was recovered from a drainage ditch at a nearby production nursery that was associated with the garden center site. Then, in December 2008 and again in March 2009, the pathogen was detected in bait leaves deployed in a stream outside this nursery.

Mississippi – *P. ramorum* was baited from a drainage ditch outside a nursery in December 2007. Subsequently, it was recovered from this ditch several times in 2008 and 2009 and a creek into which the ditch drains (using PCR on bait leaves) on multiple occasions from January 2008 through early 2009. Streamside vegetation surveys outside of the nursery resulted in PCR positives on two APHIS official host and associated host plant (HAP) (http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/) samples in December 2007 and February 2008. To date, this is the only site where streamside vegetation surveys have detected *P. ramorum* on vegetation samples.

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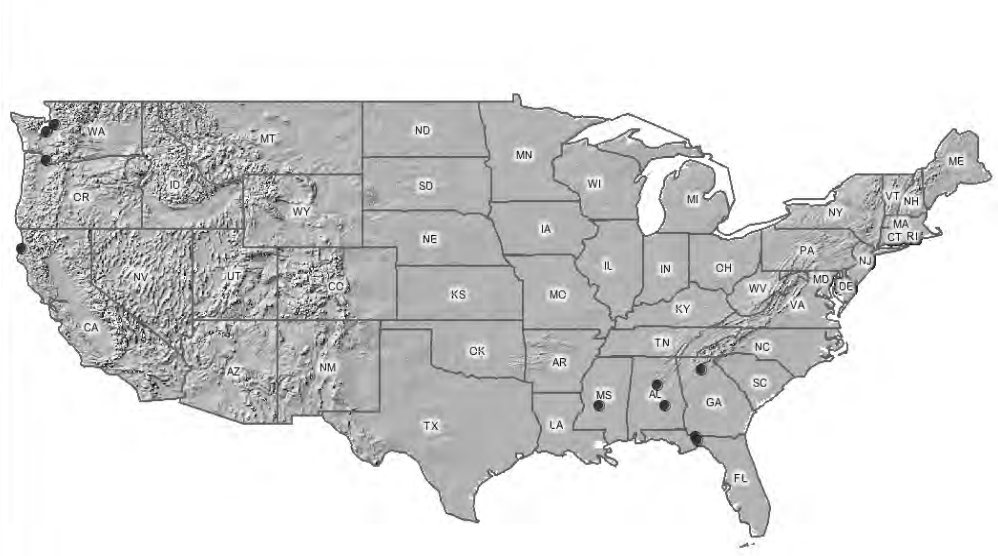

Figure 1—Locations where *Phytophthora ramorum* has been detected in waterways associated with nurseries.

Alabama – *P. ramorum* has been detected in drainage ditches along the edges of two nursery sites. At the first site, baits were negative in 2007, but were positive in November 2008 and March 2009. At the second site, baits were negative in 2007 and 2008, but were positive in March 2009.

Georgia – *P. ramorum* was baited from water draining off of a positive nursery site between December 2008 and March 2009. Bait positives were first obtained in February 2009 from a reservoir which is used for irrigation by the positive nursery, as well as an adjacent negative woody ornamental nursery and a homeowner's association in an adjacent subdivision. A stream draining the reservoir and nursery was bait-positive just off the nursery property in March, April, and May. Additional bait positives were obtained in April from one of two streams feeding the reservoir.

California – *P. ramorum* was baited from a small coastal stream in northern Humboldt County in 2006, with repeat detections in 2007 and 2009. A second stream tested positive in 2008 and 2009. Although there is a nearby nursery that tested positive for *P. ramorum* in 2004, 2006, and 2007 and a retention pond below the nursery that has tested positive in 2007, 2008, and 2009, no definitive linkage between the positive nursery and the retention pond has been made with the positive streams.

Washington – In western Washington, *P. ramorum* was first detected in a seasonal Pierce County stream associated with a positive nursery during early 2006. Baiting has detected the pathogen downstream from the original nursery site in 2007, 2008, and 2009. In King County in 2007, *P. ramorum* was detected in a retention pond outside of a positive nursery and a river into which the pond flows. The 2007 river positive bait site was positive again on two separate occasions in 2008. Extensive baiting of the river and retention pond during the first 6 months of 2009 has resulted in multiple positives at eight bait sites along the river and the retention pond. Genotyping data suggests that more than one source of inoculum may have contaminated this river. The river drains a watershed where a number of positive nurseries have been found and there are numerous, recently landscaped sites along the river. The most recent water detection in Washington occurred as the result of CNP activities at a positive nursery in Clark County in southwest Washington. Bait samples in a retention pond on this nursery and in a culvert on a drainage ditch running out of the pond at the lower property

edge, over 100 yards below the pond, tested positive in 2008. The retention pond tested positive again in 2009.

At the 2008 California Oak Mortality Task Force annual meeting, Steven Oak summarized a number of challenges relating to *P. ramorum* in rivers and streams. These included “the scale and connectivity of waterways, epidemiology unknowns, the ‘nature’ of riparian areas, treatment options, and regulatory gaps”.

The increased detection of *P. ramorum* in waterways associated with nurseries increases the risk that this pathogen will become established in urban landscapes. Likely pathways for *P. ramorum* to spread from contaminated waterways to vegetation include seasonal flooding and the use of water for irrigation. To date, *P. ramorum* has been detected on streamside vegetation at only one site. The spread of the pathogen from the stream to vegetation at this site appears to have resulted from direct exposure of susceptible host tissue to contaminated water during high stream flows. Diseases caused by *Phytophthora* spp., including *P. ramorum*, can also be spread by irrigating plants with contaminated sources of water. Currently, this appears to be a potentially significant issue at the Georgia and King County, Washington sites, where various entities have permits that allow them to use the contaminated water for irrigation purposes.

Efforts to reduce the risks associated with *P. ramorum* in waterways are hampered by a number of unanswered questions relating to the biology of this pathogen in this environment. For example, how is *P. ramorum* persisting and what is it sporulating on in the seasonal stream in Pierce County, Washington? Inoculum from a positive nursery spread into this stream during a flood event in early 2006. Although the nursery has tested negative every year since completion of the CNP in 2006 and the stream goes dry during the summer, *P. ramorum* has spread downstream from the initial positive bait site by the nursery and the stream has tested positive each year since 2006. Another question relates to inoculum thresholds required for infection of plant tissues during flooding episodes or when untreated contaminated water is used for irrigation.

Management of *P. ramorum* in waterways starts at the nursery. Educational programs are needed to increase the use of best management practices that are known to be effective in reducing development of *Phytophthora* diseases. This should include approaches, such as the use of biofilters, to reduce the risk that water leaving the nursery site contains viable inoculum. At this point it appears there are limited, if any, mitigation options for eliminating *P. ramorum* once it gets into a stream or river. It may be possible to eliminate inoculum in standing water in nurseries by treating it with algaecides or other chemicals, but these are not likely to be effective or environmentally acceptable in streams or rivers. The potential risk of spreading inoculum of this pathogen in irrigation water can be reduced by treatment of the water prior to irrigation.

Based on the growing number of sites where *P. ramorum* has been detected in waterways, increased monitoring of streams and rivers in areas where positive nurseries are located would provide a better understanding of the importance of this pathway in the overall spread of *P. ramorum* from nurseries to the urban environment. In areas such as the Puget Sound region of western Washington, where various state, community, and environmental groups are already monitoring streams and rivers, it may be possible to partner with these groups to expand these programs to include *P. ramorum*. Such a program might be patterned after Murdoch University’s "Fishing for *Phytophthora"* program to monitor and catalogue *Phytophthora* species in western Australia's rivers, streams, dams, and estuaries (http://www.fishingforphytophthora.murdoch.edu.au/). This would increase the level of monitoring activity beyond what is currently being done under the USDA FS national early detection stream baiting survey program and provide an excellent opportunity to increase public awareness about invasive pathogens such as *P. ramorum*. In conjunction with an increase in monitoring activities, an increased effort to genotype isolates and DNA samples

from nurseries and waterways would help clarify the origin of the inoculum that is spreading into waterways.

Finally, there are several regulatory issues that need to be addressed to reduce the risk that *P. ramorum* will spread from waterways to vegetation in the landscape. One issue is the current USDA APHIS concept of regulating "Diseases" caused by *P. ramorum*, but not the "pathogen." This results in questions relating to the roles and responsibilities of the various federal and state agencies in dealing with contaminated waterways. Another emerging issue relates to the notification of property owners who use water from contaminated waterways for irrigation. For example, Washington State Department of Ecology records (May 2009) indicate that 46 entities have water rights on the positive King County River. This includes a diverse group of landowners including farmers, a golf course, a turfgrass sod farm, municipalities, a church, and banks. A total of 35 permits specify the use of water for irrigation of various agricultural and horticultural crops, turf, and landscapes as well as newly established riparian plantings along the river. The total irrigated area listed by 31 of the entities is about 2,750 acres. Without some type of notification and educational program, property owners who pump water out of contaminated waterways may be inadvertently spreading *P. ramorum* over fairly large areas. This would counteract current efforts to contain the spread of *P. ramorum* and increase the risk of quarantine action by regulatory agencies.

Acknowledgments

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Emerging *Phytophthora* Species and Related Issues

Distribution and Severity of Alder *Phytophthora* in Alaska[1]

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Abstract

In Alaska, an unprecedented dieback and mortality of *Alnus incana* ssp. *tenuifolia* has occurred which stimulated an effort to determine causal agents of the disease. In Europe, similar dieback and mortality of *Alnus incana* and *Alnus glutinosa* has been attributed to root rot by a spectrum of newly emergent strains in the hybrid species *Phytophthora alni.* The variable hybrids of *P. alni* were grouped into three subspecies: *P. alni* ssp. *alni* (PAA), *P. alni* ssp. *multiformis* (PAM), and *P. alni* ssp. *uniformis* (PAU). From 2007 to 2008, we conducted a survey of *Phytophthora* species at 30 locations with stream baiting as used in the 2007 national *Phytophthora ramorum* Early Detection Survey for Forests in the United States. Additionally, *Phytophthora* species from saturated rhizosphere soil beneath alder stands were baited *in situ* using rhododendron leaves. We discovered PAU in rhizosphere soils in 2007 at two sample locations in unmanaged stands hundreds of miles apart, on the Kenai Peninsula and near Denali National Park. PAA was reported to be the most aggressive and pathogenic to alders and PAM and PAU were significantly less aggressive than PAA, though still pathogenic. To ascertain whether PAU was of restricted distribution due to recent introduction, or widespread distribution, we extended the survey in 2008 to 81 locations. Intensive sampling was conducted at five alder stands exhibiting dieback and 10 alder genets per location were excavated to expose nearly the entire root system for evaluation of the severity of root rot, ELISA detection of *Phytophthora* in diseased roots, and isolation of *Phytophthora* species. At intensive sites, four bowls each containing 500 ml samples of saturated rhizosphere soil were baited by floating three detached leaves of *Rhododendron* spp. for a 2-week period. Leaves were rinsed and sealed in plastic bags and shipped to the laboratory where leaf tissues were placed in PARPH-V8 agar selective for *Phytophthora* spp. *Phytophthora* spp. were identified from DNA sequence of the ITS-rDNA region. The survey yielded some species newly reported for the U.S., including *P. aff. gallica,*and an undescribed species in Clade 8C closely related to *P. ramorum* and *P. foliorum*, and other undescribed species. The Clade 8C species was of restricted range in our isolations, and all 20 isolates were from one location. The species was of interest to researchers developing systems for detection of *P. ramorum*. Thirty-three isolates of PAU were identified out of approximately 600 isolated and sequenced *Phytophthora* spp. PAU was collected from 11 geographically distributed stands. Only one isolate was obtained from bait floating in a water course (the Tanana River) out of 81 watercourses sampled. Soil isolates were from four plots in southcentral Alaska along the Kenai and Russian Rivers, and seven plots in the interior, including a plot in Fairbanks, three plots between Delta Junction and Fairbanks along Highway 2, two between Slana and Tok along Highway 1, and one near Denali National Park on Highway 3. PAU was widely distributed and difficult to isolate. Severity of root rot was low, with less than one diseased root discovered per genet, on average. Root rot does not appear to be a significant contributor to the dieback and mortality of alder in Alaska.

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Introduction

Species of alder (*Alnus*) were exhibiting a dieback and mortality more severe and extensive than has been recorded in history in Europe and North America. Study of the mortality in Europe was well underway by 1993 (Gibbs and others 1999) and it was usually described as exhibiting long linear cankers on stems caused by *Valsa oxystoma* Rehm 1875, and root rot caused by *Phytophthora* species. *Valsa oxystoma* is prominent in the pathology literature in Europe, having a long association with, and periodic and extensive mortality of *Alnus* (Tabeuf 1895). The stem canker, though dramatic, has been in recent times discounted as being a primary pathogen in the disease etiology of alder mortality in Europe. No clear explanation for discounting the stem canker, however, has been given. Dieback was often recorded associated with drainages having ephemeral or variable water flow which suggested water stress was a predisposing environmental factor (Webber and others 2004). Various additional episodes of dieback and mortality of alders have been documented over the years, especially in Europe (Cech and Hendry 2003). These include damage due to hydrological extremes.

In Europe, the common alder, *A. glutinosa*, and other alders, primarily *A. incana* ssp. *incana*, were exhibiting *Phytophthora* collar rot, root rot of fine roots, and tar spots on stems with dieback. Collar rot and tar spots were above ground symptoms associated commonly with *Phytophthora* diseases of trees. Tar spots were locations where a break in bark overlying infected cambium exudes a black liquid, like those observed in sudden oak death (SOD), caused by *Phytophthora ramoum*. These symptoms have been associated with the newly emergent species of *Phytophthora*, *P. alni* Brasier and S. A. Kirk 2004, which was the causal agent of the lethal root and collar disease of alder species in Europe. In Europe, alder *Phytophthora* was well documented as a lethal root and collar disease of alder in the United Kingdom (U.K.), France, Germany, Austria, Hungary, Italy, and the Netherlands. Considerable research has followed the discovery of the newly emergent alder *Phytophthora* because it was found to have arisen from hybridizations between other *Phytophthora* species (Brasier and Kirk 2001, Brasier and others 2004). *P. alni* has three variants which vary in their virulence and pathogenicity. *P. alni* ssp. *alni* (PAA) appears to be the most aggressive and pathogenic to *Alnus* species. The other two, *P. alni* ssp. *uniformis* (PAU) and *P. alni* ssp. *multiformis* (PAM), appear to be significantly less aggressive than PAA, though still considered pathogenic. The PAA variant was typically considered the primary agent killing alders in Europe. PAU and PAM variants were not well understood for their role in causing alder mortality. PAU has been detected across Europe and now was detected in Alaska. PAU was often found in soil, asymptomatic plants, and areas where PAA does not occur. Ioos and others (2006) suggested that PAU and PAM might have existed for a long time on or in the vicinity of alder trees before the recent emergence of large-scale death of alder in Europe. Thus the occurrence of PAU or PAM in the past might not have been noticed because of the lack of conspicuous symptoms or death of whole trees.

Of particular interest was that the parent species in the hybridizations were not pathogenic on alder. For hybridizations to occur in nature and result in a jump to a new host was of great interest to plant pathologists studying plant epidemics caused by newly emergent or recently introduced plant pathogens, such as *P. ramorum*.

Beginning in 2000 in North America, widespread and serious branch dieback and mortality of thinleaf alder, *Alnus incana* ssp. *tenuifolia*, was reported by land managers and others in the southern Rocky Mountains and, beginning 2003, in Alaska (Worrall 2009). Thinleaf alder ranges from the Arctic south to Arizona, and from the Pacific Coast east to central Alaska and the Rocky Mountains (Furlow 1979). In Alaska it may occur near sea level, while in the southern Rocky Mountains it was limited to higher elevations (approximately 3000 m) and riparian areas. Its primary value was in stabilizing soils and in shading and cooling streams, thereby improving fish habitat. It was a keystone nitrogen fixation species with the *Frankia* symbiont. In Alaska, less serious canker and dieback was observed in *A. sinuata* and *A. crispa*, species that more commonly occur in highlands and in the interior.

The widespread and serious branch dieback and mortality increasingly raised concerns about the future of the alder riparian ecosystem. We became involved as collaborators in studies to quantify the extent and severity of dieback and mortality in Alaska and the southern Rocky Mountains from southern Wyoming to northern New Mexico, to identify which pathogens might be potential causal agents of disease, while others assessed the remaining potential direct and indirect causal factors of the epidemic. A major impetuous throughout the study was the concern whether an introduced pathogen was spreading and becoming established in North America.

Figure 1—Typical canker on stem of *A. incana* spp. *tenuifolia*, common in stands with dieback and mortality (photo by J.J. Worrall).

The broad range goals of our 3 years of research were to improve our understanding of the cause of alder mortality in Alaska by documenting symptoms, symptom severity, and signs of disease on alder suffering dieback and mortality; compare them to those in healthy stands; isolate and identify plant pathogens; and participate in completing Koch's postulates with select pathogens. Throughout the widespread mortality, stems of the trees have exhibited narrow linear cankers, approximately 100 cm long by 2 to 7 cm wide (fig. 1). The cankers have been correlated with the dieback and mortality (Worrall 2009) and the canker surfaces usually have been entirely covered with densely aggregated ascostromata or, less often, conidiomata.

Therefore, one objective was to isolate the pathogenic fungi growing at the advancing margins of cankers on *A. incana*, isolate from stromata formed on the cankered tissues, and identify the isolated ascomycetes using morphology and molecular sequence homology. This objective necessitated a re-evaluation of the *Valsa* spp. (anamorphs *Cytospora* spp.) pathogenic on *Alnus* spp. using phylogenetic analyses and virulence among the characterized species compared on the host.

Initially, the project was funded by local U.S. Department of Agriculture, Forest Service (USDA FS), Forest Health Protection (FHP) contracts, but as the possibility increased that an exotic pathogen of concern may have been introduced, funding came from the USDA FS Forest Health Technology Enterprise Team (FHTET). The FHTET had just completed the modeling of risk factors for introduction, spread, and establishment of *P. alni* (lead by Marla Downing and posted on the Internet at http://www.fs.fed.us/foresthealth/technology/invasives_phytophthoraalni_riskmaps.shtml). Similarly, the Europeans had established a website on the potential risk of spread of *P. alni* into non-infested regions of Europe. The better studied epidemic in Europe was used as reference in modeling the risk to North American forests, and experts consulted included T. Jung (Germany). In Europe, *Alnus* species were utilized extensively in habitat restoration where nursery stock was routinely out-planted along river banks. Therefore, the introduction and spread of *P. alni* in North America was proposed as likely originating in nurseries and spreading by interstate trade in nursery stock with establishment of the pathogen occurring following out-planting, or resulting from propagules carried by watercourses from epicenters such as production nurseries. At the time we initiated our studies, *P. alni* had not been previously found in North America, although rumors had been heard of the occurrence of an isolate tentatively referred to as *P. alni* that had been found in a survey of nurseries in Minnesota. This incident was later reported by Schwingle and others (2007), but the species could not be confirmed as *P. alni*.

During these studies we collected data on edaphic factors, stem and genet size and density, and detailed landscape features. However, these latter measurements were for use in modeling and were not reported herein. The publication of Worrall (2009) thoroughly covers investigations of many ecological factors that have or have not been correlated with the epidemic disease in the southern Rocky Mountains, and the publication by the team of Ruess and others (2009) covers some ecological features of the Alaskan epidemic.

A second objective was to isolate *Phytophthora* spp. from roots, rhizosphere soils, crowns, and tar spots of alder as well as from adjacent watercourses and wetlands. Due to growing concern among pathologists and state and federal regulatory officials that cryptic invasion by the *Phytophthora* that devastates alder in Europe may be damaging Alaskan riparian forests, a more extensive survey for alder *Phytophthora* was conducted in 2007 to 2008. *Phytophthora* species were baited and trapped from a total of 81 sites across south central and interior Alaska (fig. 2). The objectives necessitated the identification and virulence testing of *Phytophthora* spp. reported as pathogenic on alder.

Particular interest in a need to know whether particular rivers or streams might be carrying the propagules of PAU and spreading the pathogen downstream was expressed by government agents. Therefore, once PAU had been discovered, it became essential to determine its location, distribution, and whether it caused serious damage to alder. The data generated from the study should address some questions concerning the biology and ecology of the newly discovered PAU. Providing this needed information to USDA Animal and Plant Health Inspection Service (APHIS) and Alaska Division of Agriculture personnel should aid them in determining whether they should pursue specific actions in regard to the finding of PAU in Alaska. The project was designed to provide some answers to the major concerns listed below:

1. What pathogen(s) are causing the stem cankers?
2. Are the stem canker pathogens native or introduced?
3. Can Koch's postulates be reproduced with select canker pathogens on thinleaf alder?

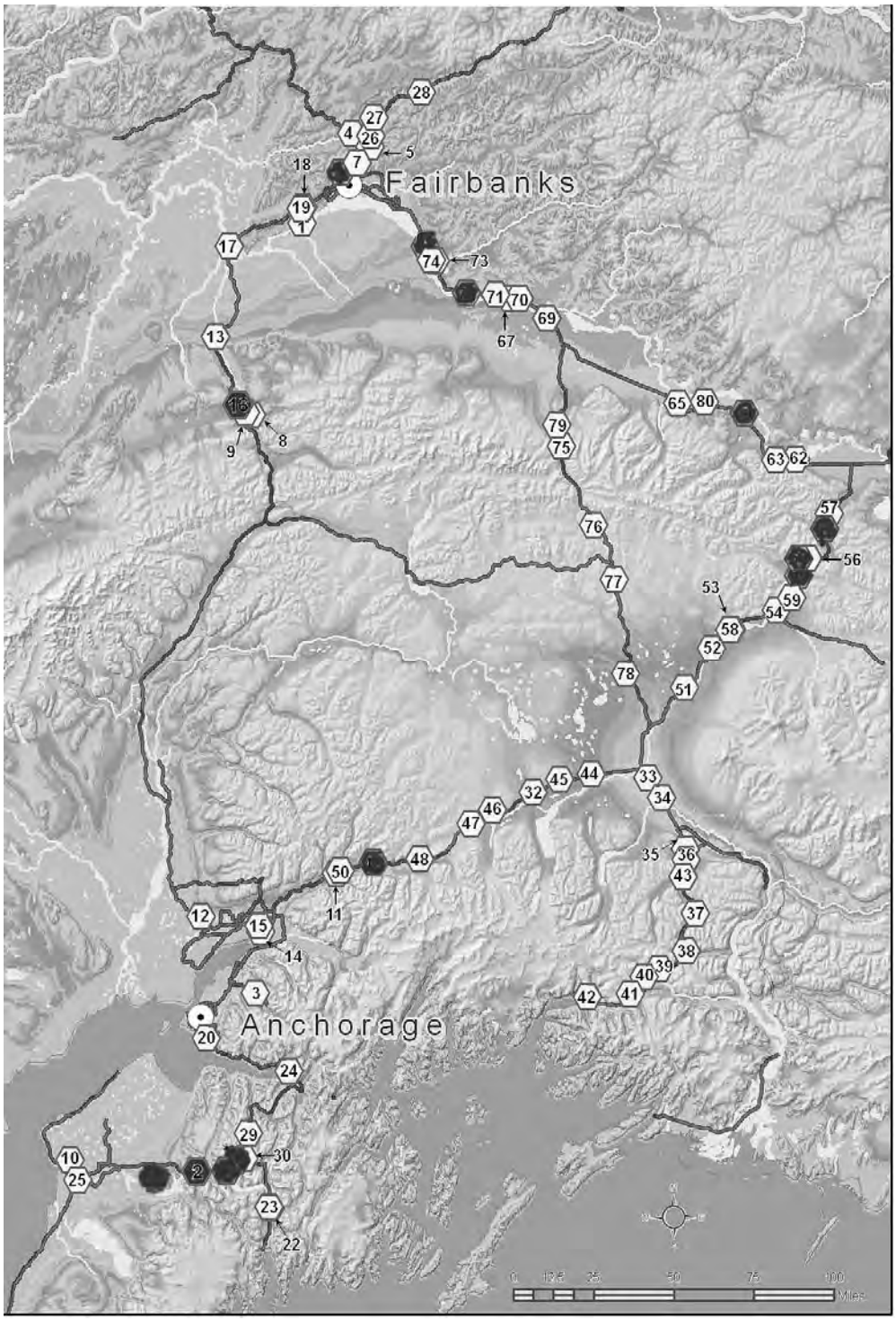

Figure 2—Map showing the distribution of extensive plots in alder stands and baited watercourses adjacent to alder stands in Alaska.

4. What was the incidence and severity of *Phytophthora* crown and root rot in alders in Alaska?
5. Is PAU causing disease on alder in riparian ecosystems in Alaska?
6. What was the distribution of PAU among alders in Alaska?
7. What streams and rivers yield PAU inoculum and may be transporting the pathogen?
8. Is PAU a recently introduced pathogen or a long established member of the Alaskan riparian ecosystem?
9. Can root rot be reproduced with PAU, a new species related to *P. ramorum, P.* aff. *gallica,* and *P. pseudosyringae* on thinleaf alder, and Koch's postulates completed?

Answering these questions concerning the biology of pathogens present in alder stands with dieback and mortality will improve decision making in the management of alder in Alaska.

Methods and Materials

Field Procedures

Extensive plots were selected from GPS identified locations established by the Alaskan USDA FS FHP team of L. Trummer. Efforts were made to target plots with a rating of "high" mortality from drive-by observations, and to target plots that would provide a well-spaced distribution. Individual sites were selected on the criteria of ease of visual location from roads once GPS coordinates were identified.

Landscape Parameters–

Once in a suitable stand, alder density was examined and an area that had sufficient alders to encompass three square subplots of 13 by 13 m [any orientation] was located using a chain. Thirty alder stands (2007 extensive plots) were evaluated for age composition by counting the number of stems greater than 12.5 cm diameter at the ground line, and those of less than 12.5 cm diameter in a central plot of 0.081 hectare ($1/20^{th}$ acre) and two microplots of 0.021 hectare ($1/300^{th}$ acre) oriented along a compass line. The slope, aspect, and form of the landscape at the central plot was measured with compass and a rangefinder/hypsometer or estimated visually. Whether the site was of a riparian, flooded, or non-flooded type; dry, wet, or seasonally wet and vertical or horizontal in proximity to the relevant waterway were measured and recorded. In the central subplot of each of the 30 extensive plots one tree was chosen (if possible, a tree partially alive that exhibited recent dieback) for soil and root studies. One pit (approximately, 60 by 60 cm) was dug to a depth of 60 cm with a shovel and a standard soil probe was used for greater penetration below 60 cm. Soil structure was evaluated, the depth of soil profile change was noted based on color and mottling, and presence of ice, gravel, or hard pan barriers to root penetration were recorded. Soil texture percent and stratification were estimated tactically and visually, and soil organic matter was estimated. Plot data was recorded in the attached Excel datalog files on the forms supplied by FHTET with some additional fields added.

Trapping *Phytophthora* Species

Phytophthora and *Pythium* species were trapped from roots, soils, and water sources using rhododendron leaves and nucleopore filtering. All references to leaves herein refer to *Rhododendron catawbiense* leaves.

Baiting Waterways

Sixteen to 20 leaves were placed in a nylon screen [window screen] envelope, 30 by 30 cm. The leaves were spaced in four rows of four to five leaves using staples to create four long pockets in the screen envelope. One edge of the envelope was wrapped around a 2.5 cm diameter PVC pipe for anchoring, and a strip of bubble wrap [about 8 by 30 cm] was stapled to the opposite end for floatation. A rope was passed through the PVC pipe and tied in a circle. Then about 10 m of 286 kg fishing line was attached to the rope and anchored to a tree trunk on shore. The envelope was tossed into the water to float. Two such envelopes of leaves, henceforth called baits, were left at each alder plot in the nearby waterway. Usually one screen of bait was positioned midstream anchored by a 2.2 kg fish weight on a 2 m tether, and the second bait floated at the shoreline without an anchor. Following about 2 weeks in the waterway, the baits were retrieved; leaves were removed and washed, packaged in sterile plastic sample bags, and periodically mailed to the laboratory triple-wrapped with a copy of permit papers (APHIS permit P526-070620-002). In instances when water temperatures were cold or incubation time was less than 2 weeks, scissors were used to wound each leaf in the envelopes to accelerate colonization by *Phytophthora* species. This methodology was described on the USDA FS SOD website (http://fhm.fs.fed.us/sp/sod/sod.shtm). The screen baits were deployed in rivers and nearby watercourses that had considerable numbers of *A. incana* ssp. *tenuifolia, Alnus sinuata* and/or *Alnus crispa* present. When suitable sites were located where dieback occurred in an alder stand, the site was established using a string chain and compass to outline a minimum of one central plot of 0.081 hectare ($1/20^{th}$ acre) and two microplots of 0.012 hectare ($1/300^{th}$ acre). The site was named and numbered. The location was logged using global positioning (GPS) in coordinates of the WGS 84 system and elevation.

In 2007, baits were floated in 30 watercourses. In 2008, baits were floated in 51 additional water courses for a total of 81 sampled locations. Baiting materials were diversified in 2008 with the addition of bearberry leaves (*Arctostaphylos uva-ursi*), alder twigs (*A. incana* ssp. *tenuifolia*) and a reduced number of rhododendron leaves in a screen.

Filtering Water From Waterways

Over 2 l of clear water from each of 30 watercourses were collected in 2007 and double bagged at each extensive plot, then placed in an ice chest for transport. That evening, the water was filtered through a 3 to 5 µm mesh polypropylene microbial filter (47 mm diameter nucleopore membrane filters). Using a 60 ml syringe, 300 ml of water was pressed through each filter and three filtrations per plot were collected for about1 l. When water-borne sediments occluded filter pores, more than three filters were employed to filter about 1 l of water. The filters were laid face down on the surface of a leaf and sandwiched between leaves in a stack. The sandwiched leaves and filters were placed tightly together in a sterile bag with distilled water and incubated at ambient temperatures. After about 2 weeks the leaves were removed and washed to remove the membrane, soil, and debris, then packaged in sterile plastic sample bags and periodically mailed to the laboratory triple bagged with a copy of

APHIS permit papers. A similar methodology is described on the USDA FS SOD website.

Baiting Rhizosphere Soil

At each extensive plot, soil was excavated from one tree for soil structure studies as described above. Soil and roots collected from the excavation were placed in plastic Ziploc® bags. The collected soil was obtained from that intermingled with roots and clinging to roots and usually included root fragments, for this reason we refer to it as rhizosphere soil. In the subsequent evening, a portion of the soil was placed in two styrofoam bowls and watered with distilled water to saturation. Roots were treated as described below. Three leaves, some wounded and some intact, were placed on the soil surface and the unit was sealed in a water-tight Ziploc® bag. The bowls were maintained at ambient temperature (from car interior to motel rooms). After about 2 weeks the leaves were removed, washed, packaged in sterile plastic sample bags, and periodically mailed to the laboratory triple bagged with a copy of APHIS permit papers.

Baiting Roots

In Alaska, collected roots were washed carefully, cut into 1 cm long segments and 6 pieces sandwiched between two leaves. This was repeated for a minimum of 24 root pieces/plot. The sandwiched leaves and roots were placed tightly together in a sterile bag with distilled water and incubated at ambient temperatures. After about 1 week the leaves were separated and washed to remove root pieces and soils, packaged in sterile plastic sample bags, and periodically mailed to the laboratory triple bagged with a copy of APHIS permit papers.

Excavation of Entire Root Systems and Intensive Plot Sampling

In 2008, five plots were chosen in the Kenai Peninsula for intensive sampling. These plots included one location where *P. alni* had been discovered in 2007, three plots along the watercourse, the Russian River, common to the *P. alni* infested plot, and a fifth plot several miles from the river near Kenai City. At these intensive plots, five live alder genets (multi-stemmed alder clumps sharing a common crown and root system) with recent dieback were chosen. The circumference of the crown was visually divided into quadrants, and four workers excavated the root system of a quadrant by removing soil to expose roots for examination (see fig. 3). For each quadrant, 10 flare roots and 10 pencil-diameter sized roots were visually assessed for disease and decay using a pen knife. The number and condition of roots exhibiting rot were recorded. Root systems with rot were assayed using recently diseased root tissues prepared for the *Phytophthora* detection ELISA diagnostic kit (Agdia Inc., Elkhart, IN 46514) and processed in the field (Timmer and others 1996). Per genet, the total number of stems > 2.5 cm, living stems, dead stems, and cankered stems (dead and alive) were recorded. A walk-by survey of every genet in the intensive plot was made in search of tar spots. All tar spots were assayed with the ELISA kit in the field. Soil from the rhizosphere of each quadrant was placed in a separate styrofoam bowl and sealed in a plastic bag until evening. In the evening, distilled water and rhododendron leaves were added to each bowl and incubated at ambient temperature for about 2 weeks. Leaves were harvested, washed, packaged, and mailed as described above. Everett M. Hansen from Oregon State University joined us during some of the excavations and searches for tar spots (below) as a mentor and collaborator.

Figure 3—Excavation of four quadrants of the root system of a multi-stemmed alder genet for examination of roots for root rot, and for sampling rotted roots for ELISA *Phytophthora*-detection assays, *in situ*.

Sampling Tar Spots and Collar Rots

Three to four observers walked through five intensive and three extensive plots searching for tar spots and above ground visual evidence of collar rot. At the same time, alders exhibiting recent dieback were examined for little leaves and yellowing foliage. To facilitate recognition of these disease symptoms in the field, this survey of symptoms was conducted using the disease leaflet of Jung and Blaschke (2001). Affected bark from tar spots and suspect collar rots were excised from the trees, and the cambium examined for discoloration. Samples with discolored cambium were tested with the *Phytophthora* detection ELISA assay. The remaining material was wrapped in rhododendron leaves and placed in a sterile plastic bag with some distilled water to encourage sporulation and infection.

Laboratory Procedures

All laboratory procedures were performed at Michigan State University (MSU).

Isolations from Leaves

Upon arrival at the lab, sample bags were placed in a locked 13 ^{o}C incubator until the investigator could open the packages and sample bags in the certified, and APHIS-approved ,microbiological biosafety level 2 hood/cabinet. A number four cork borer was used to aseptically cut disks of leaf material along the length of each leaf. The disks were pushed down into agar plates containing PARPH-V8 *Phytophthora*-selective medium. These plates were incubated at 19 ^{o}C in the dark in a locked incubator and examined at 1-week and 2-week intervals. This methodology is described in Hwang and others (2008). Colonies were categorized by appearance,

picked from the plates, and transferred individually to 5 cm Petri plates of PARPH agar overlain with a membrane of cellophane.

Canker Pathogens

From all extensive plots and a majority of the 81 watercourse sites, ascostromata on bark pieces of less than 2.5 by 2.5 cm were collected from active cankers or dead branches of *Alnus* spp. from 2006 to 2008 (APHIS permit P526P-07-07216). Cultures were isolated from the transition between necrotic and white cambium at the margins of cankers using standard methods. These included misting 95 percent ethanol on the external surfaces, brief ignition (flaming), aseptic cutting to expose the canker margin, and isolation of infected tissue pieces. Acidified malt extract agar (1.25 percent malt extract, 100 ppm streptomycin sulfate, pH 5) was used to culture the fungi. Additionally, axenic cultures were obtained by cutting fruiting bodies in half horizontally and applying a drop of water to an exposed locule. Following swelling of the gelatinous matrix, some of the spore mass was lifted and streaked across the surface of the agar medium. Actively growing colonies were purified by excising a terminal cell from an individual hypha. A terminal cell was transferred to an agar plate and subsequent cultures were derived from the hyphal tip cell. Other cultures were obtained from culture collections. The origin for collected specimens was recorded as GPS coordinates.

DNA Preparation, Sequencing, and Analysis

Hyphae were harvested from the cellophane surface for grinding and DNA extraction. Dilutions of DNA extracts were used in PCR amplification reactions of the ITS region of the ribosomal DNA operon. Standard PCR amplification methodology was employed with pairs of primers. Primers included ITS1, ITS1f, ITS5, and ITS4 for the ITS-rDNA (Gardes and Bruns 1993; White and others 1990); Bt-UP4 and Bt-1b for a portion of the β-tubulin gene (Lavésque, personal communication; Glass and Donaldson 1995); and FM75, FM77-80, FM84, FM84-85 for the COXI-COXII region (Martin and Tooley 2003). The cycling reactions were performed in a DNA Thermal Cycler (Perkin-Elmer Corp., Norwalk, CT, U.S.) using standard protocols. PCR products were sorted by size on electrophoretic gels. Appropriate PCR products were purified and submitted for autosequencing at the MSU Research Technology Support Facility. Twenty µl of each PCR product were purified by using the DNA binding resin and protocol of Wizard PCR Preps DNA purification system (Promega Corp., Madison, WI, U.S.) and used in sequencing reactions. Sequencing was performed using a *Taq* DyeDeoxi Terminator™ cycle system, the ABI Catalyst 800, and the ABI Prism 373A or 377 fluorescence sequencer (PE Applied Biosystems, Foster City, CA, U.S.) using the Big Dye fluorescent labeling sequencing kit (PE Applied Biosystems). Amplified double-stranded PCR products were sequenced independently along both strands with the primers listed above. Final sequences were compared for homology to the NCBI GenBank database using BLASTn software. Identification of *Phytophthora* and *Pythium* isolates were preliminarily based on about 760 bp of sequence homology at about 98 percent match (standard procedures). Final identification of distinct species was based on homology to two or more different gene sequences and morphological examination. The RAS-Ypt gene sequence was used as the second homologous match in confirmation of identification of isolates of *P. alni* subspecies (Ioos and others 2006). The identification to subspecies *uniformis* was by amplification and restriction enzyme digestion of sequence characterized markers, as described in the SCARS protocol of Ioos and others (2005).

Results and Discussion

During July 2007, three isolates of *P. alni* ssp. *uniformis* Brasier & S. A. Kirk 2004 (PAU) were isolated from soil beneath *A. incana* ssp. *tenuifolia* in Alaska in two of the extensive plots (Adams and others 2008a; Trummer and others 2007). The two extensive plots were in remote, unmanaged stands hundreds of miles apart: on the Kenai Peninsula and near Denali National Park. Both of these locations, however, appeared to receive common traffic from international tourists, particularly tourists seeking fishing opportunities in Alaskan rivers. The alders in the plots exhibited the dieback common to a widespread epidemic disease in Alaska and the southern Rocky Mountains, including stem cankers with abundant ascostromata. PAU were trapped from saturated rhizosphere soil baited with rhododendron leaves. Species identification of the three isolates was based on DNA sequence homology of ITS and RAS-Ypt molecules (Ioos and others 2006), subspecies identification by SCAR profiles (Ioos and others 2005), and morphology (GenBank EU371544-371553). Caducous sporangia were observed in the Alaskan isolates by Hansen and Reeser (Oregon State University); this characteristic had not been previously observed in *P. alni*. Labs of USDA FS and APHIS (S. Diehl and Z.G. Abad, respectively) have additionally confirmed the identification. *P. alni* was not recovered from the other 28 sample sites (extensive plots) in 2007.

The pathogenic fungi isolated from the advancing margins of the cankers and from most stromata have been species of *Valsa* (anamorph *Cytospora*). Symptoms of Phytophthora root rot were not evident on examination and collection of roots during excavations of the tree in each extensive plot in 2007 (Worrall 2009). Therefore, in 2008 further efforts were undertaken to observe root condition in stands that had yielded PAU.

In 2008, we discovered 30 more isolates (total = 33) of PAU. All but one isolate was trapped from baited soils, while one was trapped by alder twig bait in the Tanana River. The range of known occurrence of this oomycete has been expanded to include 11 geographically distributed alder stands or adjacent watercourses out of 81 watercourses sampled. Soil isolates were from four plots in south central Alaska along the Kenai and Russian Rivers, and seven plots in the interior, including a plot in Fairbanks, three plots between Delta Junction and Fairbanks along Highway 2, two between Slana and Tok along Highway 1, and one near Denali National Park on Highway 3. PAU was widely distributed and difficult to isolate. Discovery of the putatively exotic and invasive species, PAU, led directly to a need to confirm this species' virulence on the native *Alnus* spp. in Alaska. The PAA variant has not been found in North America. We stress that only the *P. alni* ssp. *uniformis* variant has been found in Alaska. Pathogenicity tests on *A. incana* ssp. *tenuifolia* are underway in 2009 at Oregon State University under the direction of Hansen.

Isolates of PAU were present in four out of five intensive plots, and in all the intensive plots that were within the tributaries and drainage basin of the Russian River corridor in the Kenai Peninsula. The one intensive plot several km from the Russian River drainage did not yield any isolates of PAU. The frequency of isolation of PAU among the *Phytophthora* trapped in the four PAU-positive intensive plots was low and ranged from 0.6 to 14 percent (0.6, 10, 11, and 14 percent). This distribution along a river corridor in the south central state, and the isolation of PAU directly from one river in the interior, supported the supposition that a low level of

PAU inoculum may be spread in the rivers of south central and interior Alaska where the dieback and mortality has been observed.

Results of evaluation of the condition of stems and roots of alder genets in the intensive plots are summarized in table 1. Excavations of total root systems in 2008 revealed that symptoms of root rot, collar rot, and tar spot were rarely present at the intensive plots and examined extensive plots. Severity of root rot was low with less than one diseased root discovered per genet, on average (table 1). Armillaria root rot was encountered on four genets in the intensive plots and more frequently at one extensive plot (Potter Marsh). Rotted roots occasionally tested positive with ELISA for *Phytophthora*, but positive assays were limited generally to approximately one root (about 1 cm diameter) per genet with one to three positive assays per intensive plot (10 genets/plot). The scarcity of root rot in flare roots, pencil diameter roots, and *Phytophthora* isolations from fine rootlets provided evidence that *Phytophthora* root rot was unlikely to be a significant contributor to the dieback and mortality of alder in Alaska.

Table 1—Disease symptoms in intensive plots (mean of 10 genets/plot)

Intensive Plot	% Dieback	% Valsa	% Root Rot	% Collar Rot	% Tar Spot	% Sprouts	% Little Leaf
Cooper Landing	31	50	0.5	0	0	80	10
Quart Creek	40	73	2.5	20	30	80	0
Daves Creek	48	72	0.5	0	20	90	0
Kenai City	64	31	1	0	0	100	0
Hidden Lake	48	71	1.5	10	0	100	0
Average	46	59	1.2	6	10	90	2

Tar spots were rare and difficult to find in all plots. Crown rots were also rare. ELISA assays did not indicate that *Phytophthora* was likely present in cambium underlying tar spots or crown rots. Isolations from tar spots yielded a variety of ascomycetes, including *Cadophora* spp., *Cryptosporella suffusa*, and *Hypocrea* spp. Crown rots yielded a variety of ascomycetes, including *Diatrypella* sp. and *Phoma* spp., but not oomycetous *Phytophthora* spp.

The *Phytophthora* spp. and *Pythium* spp. isolated during the riparian surveys in Alaska and identified by sequencing the ITS region of the nuclear ribosome repeat unit are tallied in table 2 (Adams and others 2008b). The four methods of trapping the plant pathogenic water molds each yielded more propagules of the species of *P. gonapodyides* than any other species. The greatest diversity of species was trapped by baiting the saturated rhizosphere soil, and the least by incubating rootlets with bait leaves. The nucleopore filtering method did not yield the quality of results expected from literature reports (Hwang and others 2008). In part, this latter result may be due to the cold temperatures of the watercourses in the Alaskan north from late June through July. After July, salmon runs entice bears to move toward the watercourses and among the alder stands, so field work is suspended.

Table 2—Number of isolates of *Phytophthora* and *Pythium* species from Alaskan surveys

Alaskan Species Beneath Alder	2007	2008	Total
Phytophthora gonapodyides	90	83	173
Phytophthora sp. "hungarica" (isol. UASWS032)	5	29	34
Phytophthora alni subsp. *uniformis*	3	30	33
Phytophthora cactorum	1	3	4
Phytophthora pseudosyringae	0	11	11
Phytophthora sp. "near-ramorum" n. sp.	0	15	15
Phytophthora aff. *gallica*?	0	2	2
Phytophthora inundata	1	0	1
Phytophthora (no known relatives) n. sp.	0	2	2
Phytophthora sp. in other unnamed groups	2	14	16
Phytophthora rosacearum	4	12	16
Phytophthora megasperma sensu stricto (isol. 97-104)	15	48	63
Phytophthora megasperma sensu lato			
Phytophthora sp. "Missaukee" (isol. P47)	0	10	10
Phytophthora sp. "Missaukee" (isol. P79)	0	10	10
Phytophthora sp. "Missaukee" (isol. P61)	0	3	3
Phytophthora sp. 4, "Missaukee" FFL-2008	0	20	20
Phytophthora sp. raspberry group	0	1	1
Phytophthora sp. “SalixSoil” (isol. WD54a,b)	36	15	51
			465
	2007	2008	Total
Pythium sterilum	17	8	25
Pythium macrosporum	5	41	46
Pythium undulatum	12	7	19
Pythium pachycaule	1	6	7
Pythium anandrum	0	5	5
Pythium lutarium	0	5	5
Pythium delawari	0	3	3
Pythium boreale	1	0	1
Pythium sp. in other unnamed groups	48	8	56
			170
		Grand total	636

A considerable number of isolates in Alaska were identified as belonging to unnamed species (table 2). Most of these belonged to related groups of *P. megasperma s. l.* (Hansen and others 1986, 2009). The lack of an assignable name was the result of a paucity of distinctive morphological characteristics available for taxonomists to use in differentiating among them. Many of the *Pythium* species were new reports for the Americas, and several have just recently been described beneath alders in European studies (table 2).

The unnamed groups of *Phytophthora* species were referred herein to the isolate number accessioned in the NCBI GenBank database to which they most closely match in DNA sequence homology based on BLAST searches. Similarly, the four unnamed *Pythium* spp. were best matched to isolates P15845, 93-70P, UASWS018, 824b, or B07. *Pythium* spp. were similar to those we trapped using the same methods in 2007 in the southern Rocky Mountains (data not shown).

A putative new species of *Phytophthora* previously unknown to science related to *P. ramorum* was found in Alaska during riparian *Phytophthora* surveys and confirmed in November 2008 (Trummer and others 2008). The Alaska *Phytophthora* was sent to the USDA APHIS Plant Protection and Quarantine (PPQ) Plant Safeguarding and Pest Identification, National Identification Services Molecular Diagnostics Laboratory and also to Hansen, Oregon State University. Both labs have analyzed DNA sequences of the ITS-rDNA and COX I-II regions and reached the conclusion that the unique sequence identifies this isolate as a member of Clade 8C, the *P. ramorum/P. lateralis* clade, but as a new member. The discovery of a new *Phytophthora* species at the Quartz Creek site is especially interesting because its sequence aligns closest to several other tree pathogens of importance, including *P. lateralis*, a root pathogen of Port-Orford-cedar; *P. hibernalis*, a citrus pathogen that also can cause cankers on Port-Orford-cedar; *P. foliorum*, a new species of unknown virulence and host range; and *P. ramorum*, an oak pathogen. It was agreed that the new *Phytophthora* isolates were unique and worthy of pursing formal description. Since the new species was in the group (Clade 8C) which contains *P. ramorum*, it may be useful in improving the accuracy of detection assays for *P. ramorum*. Testing of the original nested primers for detecting *P. ramorum* (Garbellotto 2003) revealed that an amplified PCR product of the appropriate size occurred with the Phyto1-Phyto2 primer pair and the Phyto-3-Phyto-4 primer pair (fig. 4).

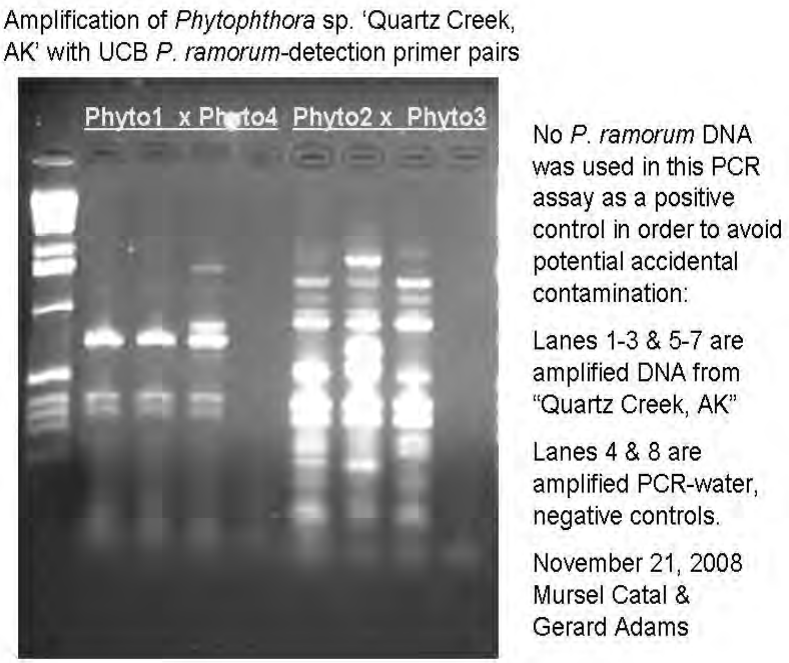

Figure 4—The original primer pairs designed to be species-specific in detection (amplification) of *P. ramorum* amplified the DNA of the new species and, in the past, might have given a false-positive identification which would not be clarified well by sequencing homology comparisons.

Further testing of the new species with DNA methodology developed for species-specific detection of *P. ramorum* revealed that the TaqMan real-time qPCR protocol in current use (Bilodeau and others 2007) did not give false-positive recognition of the new species. Despite the DNA amplification of the new species by the primer pair, the probe oligonucleotide did not anneal to the amplified sequence of the new species (fig. 5). We tentatively refer to this new species as *P*. "near-ramorum," herein.

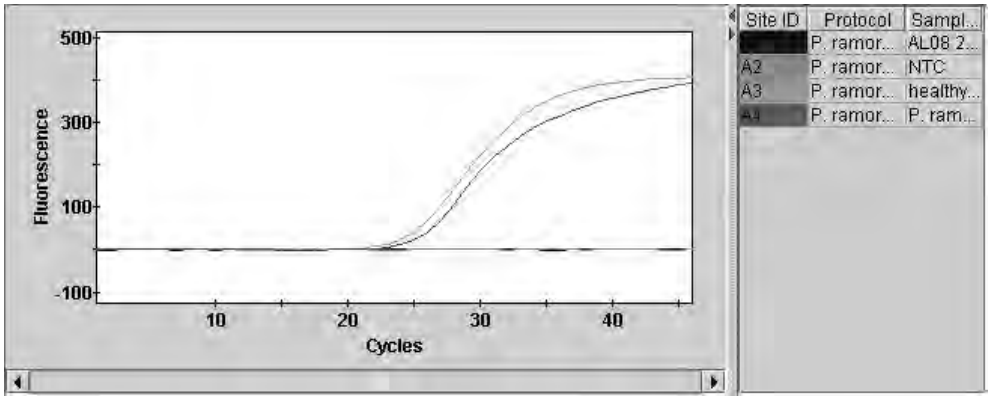

Figure 5—Michigan State University (MSU) Diagnostic Services sample 20084515. Results of real-time PCR for detection of *P. ramorum* using a TaqMan system on the Cepheid SmartCycler per APHIS work instruction WI-B-T-1-6. The new species did not show a false-positive determination in the current *P. ramorum* detection protocol because the internal probe did not anneal to the amplified product (Assay by Jan Byrne, MSU).

Isolates of *P*. "near-ramorum" came from the intensive plot along Quartz Creek, Kenai Peninsula, Alaska, where we had excavated nearly the entire root systems of genets while looking for root rot in 2008. An example of the condition of a genet that yielded soil isolates of *P*. "near-ramorum" at this location follows. Genet 3 had 40 percent top dieback and had *V. melanodiscus* cankers. It also had six roots with rot, four with Armillaria root rot and decay, and two with rot of unknown cause and no decay. While ELISA test kits for detection of *Phytophthora* were not used on genet 3, several other genets at the Quartz Creek site were positive for *Phytophthora* spp. The unusual feature of this discovery is that 15 isolates of *P*. "near-ramorum" from several genets were readily recovered at this site, but not one isolate was found in any other of the 81 widely distributed sites containing alder stands. The restricted distribution of *P*. "near-ramorum" might be suggestive of a recent introduction of an exotic species. The origin of this species might be an important keystone in unraveling the geographic origin and evolution of *P. ramorum.*

More than 50 canker isolates from Alaska were selected for DNA sequence comparisons. Alaskan isolates were referred to by the name of the river, creek, or marsh and a strain number, for example, LittleSusitnaRiver5 (often abbreviated). Sequences of additional isolates from *A. incana* from other geographic regions in North America were included in the analyses. Sequences of *Valsa* spp./*Cytospora* spp. from other parts of the world, culture collections, and from other hosts were included in phylogenetic analyses. Many of the latter have been characterized in previous studies (Adams and others 2005, 2006).

Alder mortality and dieback of *A. incana* ssp. *tenuifolia* was often associated with extensive cankers with *V. melanodiscus* G.H. Otth 1870, *Cryptosporella suffusa* (Fr.:Fr.) L.C. Mejia & Castl. 2008, *Melanoconis alni* (primarily seen in *A. sinuata* and *A. crispa* stands), or other ascomycetes on stems. Most canker isolates from Alaska fell into two distinct clades in each of the phylogenetic analyses of individual gene data sets. The majority of isolates were identified as *V. melanodiscus* and the remainder as *V. diatrypoides*. In contrast, Michigan isolates from *A. incana* ssp. *rugosa*, and a reference culture obtained from cankers on *A. incana* ssp. *tenuifolia* in Oregon, by Filip and others (1992), clustered with the species-complex represented primarily by *Valsa nivea* (Hoffm.) Fr. (= *Leucostoma niveum* (Hoffm.) Höhn.). However, some Michigan isolates clustered within the sister taxon, *Valsa leucostoma* (Pers.) Fr. (= *Leucostoma persoonii* (Nitschke) Höhn.). Serious dieback and mortality are not present in alder stands in Michigan and Oregon. In virulence trails on bolts of *A. incana* ssp. *rugosa*, only *V. melanodiscus* and *V. diatrypoides* were virulent (Adams, unpublished data). Cytospora cankers were also prevalent in the European disease situation, but in Alaska *Cytospora* may be the primary pathogenic agent involved in the dieback following predisposition of the host by unknown environmental stresses. Cankers that resulted from inoculations with *V. melanodiscus* isolates (Stanosz and others 2008) resembled naturally occurring cankers in the stands exhibiting dieback and mortality. Cankers were longitudinally elongated with sunken and necrotic bark, the underlying cambium was discolored, and margins between diseased and healthy tissues were discrete. Fruiting occurred occasionally in the necrotic bark. Analysis of variance of log transformed data revealed strong support for effect of location ($P = 0.04$), but not that of isolate ($P = 0.12$) or interaction ($P = 0.20$) on canker length (Stanosz and others 2008). Re-isolation of the inoculated pathogen yielded colonies consistent with *V. melanodiscus* from a majority of chips from margins of cankers on inoculated stems, but not from control stems. The fungus was isolated in pure culture from each canker and Koch's postulates completed (Stanosz and others 2008).

The finding of more isolates of *P. alni* ssp. *uniformis* remains perplexing. Thorough excavations of 50 alder root systems in Alaska in summer 2008 (fig. 3) revealed little evidence of root disease. Diseased roots seldom tested positive for *Phytophthora* spp. by ELISA assays. Examination of plants for other symptoms of *Phytophthora* disease, such as collar rot and tar spot on stems, has yielded scarce symptoms.

There were many unanswered questions, particularly on the origin of these organisms, their ability to cause disease, and the corresponding host ranges. Further study was needed to determine whether these organisms were introduced, how they might have been introduced, and if so whether these organisms have been causing disease on alders or other plant species in Alaska. Current research should provide a detailed estimation of the population genetics of the pathogen, *V. melanodiscus*, that was causing disease and mortality of alders in Alaska riverine forests. Similarly, we have begun studies of the population genetics of the pathogen *P. alni* spp. *uniformis.* The study will identify variation in genetic markers among the populations and verify whether the populations show high diversity characteristic of native populations or low diversity characteristic of recent invasive introductions. These results will support or refute the hypotheses concerning whether the alder mortality was caused by an invasive species, or by predisposing environmental conditions and an Alaskan native pathogen. The project should also verify whether a *P. alni* has been introduced

from Europe. Additionally, current research spearheaded by E.M. Hansen, P. Reeser and laboratory colleagues at Oregon State University will demonstrate whether PAU or other *Phytophthora* species are capable of causing root rot of alder. The results of the study have improved our understanding of the cause of alder mortality.

We do not know the host range of this new *Phytophthora* species in Alaska other than the fact that it can infect rhododendron leaves. Rhododendrons do not occur in the native environment in Alaska, though several other ericaceous hosts were present. Perhaps the new *Phytophthor*a sp. and PAU have co-existed benignly in Alaska beneath alder and have not been noted due to the lack of surveys or the lack of conspicuous symptoms or death of alder or other associated plant species. Conversely, further analysis of the isolates may reveal that the organisms could have been introduced into America on wading boots or other equipment of European fishing tourists or other travelers. Risk models of invasive species apparently have not considered this potential route to introduction of forest pathogens.

Research conclusions on these topics should improve decision-making in the management of alder in Alaska. Data from this study can be used to refine models of decline and mortality used in the National Forest Risk Map project.

Conclusions

1. Are the stem canker pathogens native or introduced?
   Preliminary AFLP analysis shows high diversity and sexual reproduction.
2. Can Koch's postulates be reproduced with the canker pathogens?
   Yes, three labs have demonstrated pathogenicity.
3. What was the incidence and severity of root rot in alders in Alaska?
   Less than 0.6 percent of roots per genet (excluding *Armillaria* spp.).
4. What was the distribution of PAU among alders in Alaska?
   Select locations along most major highway routes.
5. What streams and rivers may be transporting PAU?
   The Russian, Kenai, and Tanana Rivers.
6. Is PAU a recently introduced or a long-established pathogen?
   Yet uncertain, but it was not introduced from nurseries, as predicted.
7. Is PAU causing disease on alder in riparian ecosystems in Alaska?
   Probably not significant.
8. Can alder root rot be reproduced with PAU, *P.* aff. *gallica,* and *P. pseudosyringae*?
   We may know soon (via E.M. Hansen).
9. Should we continue to be concerned about *P. alni*?
   Vigilance is needed concerning introduction of *P. alni* ssp. *alni.*

Acknowledgments

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Detection of Possible *Phytophthora pinifolia* Infection in *Pinus radiata* Green Sawn Timber Produced in Chile[1]

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Abstract

A new needle blight disease was observed on *Pinus radiata* in Chile during 2004. The disease, known in Chile as Daño Foliar del Pino (DFP), stretches southward from the Arauco to Valdivia Provinces, and was present over an area of about 60 000 ha in 2006, with different levels of intensity. The disease is typified by needle infections and exudation of resin at the bases of the needle brachyblasts. Only *P. radiata* trees have been affected by DFP. Other *Pinus* species in the area, such as *P. pinaster*, remain healthy. Isolations from infected needles on selective media have consistently yielded a *Phytophthora* sp. DNA sequence comparisons for the ITS rDNA and *cox* II gene regions, and morphological observation, showed that this oomycete represents a previously undescribed species, which has been named *Phytophthora pinifolia* (Durán, Gryzenh, and M.J. Wingf). Research is underway to fully elucidate the life cycle of *P. pinifolia* and to develop appropriate management strategies on Chilean pine plantations.

Despite being an aggressive pathogen and an aerial *Phytophthora*, *P. pinifolia* is phylogenetically closely related to other *Phytophthora* spp. that are mildly pathogenic and normally associated with soil and roots. Pathogenicity trials with *P. pinifolia* have clearly shown that it is pathogenic to *P. radiata* and causes rapid death of the succulent apical parts of young plants. *P. pinifolia* is the first *Phytophthora* sp. known to infect needles of a *Pinus* sp. and its aerial habit is well-matched with the occurrence and symptoms of DFP in Chile.

To understand the behavior of *P. pinifolia* in green timber, a study was conducted to determine the possible presence of *P. pinifolia* in green sawn timber produced from trees that had been exposed to infection by the pathogen for at least 4 years. Green timber from the infected trees, and green wood samples exposed to *P. pinifolia* inoculum, were analyzed by making extensive isolations on *Phytophthora*-selective media. In addition, fluorescence microscopy was used to observe the possible presence of structures of the organism and PCR was conducted using species-specific primers developed for *P. pinifolia*.

Results of the study showed that the green sawn timber taken from trees infected by *P. pinifolia*, or even green timber exposed to contaminated pine plantations, showed no evidence that the pathogen can survive or develop in green wood. These results provide strong evidence that green sawn timber produced from *P. radiata* trees infected with *P. pinifolia* is free of the pathogen and that it can be exported safely without any specific treatment against *P. pinifolia*.

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Introduction

Daño Foliar del Pino (DFP) was observed for the first time during the year 2004 affecting *Pinus radiata* plantations located on the coast of the Bio-Bio Region in Chile. The disease, caused by *Phytophthora pinifolia*, corresponds to a new species of pathogen recently isolated from needles from *P. radiata* plants and trees (Durán and others 2008, R. Ahumada, Bioforest S.A., personal communication).

The disease is characterized by the appearance (in late autumn and early winter) of translucent bands seen at first glance as black bands on pine needles, and later turning into a generalized discoloration of foliage, resulting in a greyish aspect of the crown, which turns reddish brown at the end of spring as a consequence of the necrosis of the affected foliage. In addition to the damage of the foliage, damage occurs in the succulent tissue of branches and stems of young trees. In 1-year-old plantations, the attack normally causes the death of plants, whereas in 2- to 6-year-old plantations, needle damage is observed without the occurrence of mortality. In adult plantations (above 6 years), DFP is mostly seen in the foliage. No clear evidence of an effect on stems or branches of attacked trees has been detected to date.

Currently, the symptoms may be observed in plantations of all ages which grow in coastal zones of the Province of Arauco, Region of Bio-Bio and the Regions of La Araucania and Los Rios, as well as in some young plantations from the Region of Maule.

Objectives

This study was conducted over the last 4 years in order to determine if *P. pinifolia* is present in green sawn lumber from *P. radiata* trees with high levels of damage by DFP, and to evaluate if *P. pinifolia* may contaminate and develop on the sawn wood when exposed to pathogen inoculum, and thus be a source of disease spread.

Materials and Methods

The study was developed in two stages. The first consisted of evaluating and monitoring for traceability specimens of green wood obtained from trees in plantations severely affected by DFP for the last 4 years. The second stage consisted of the artificial inoculation of specimens of green wood with *P. pinifolia* under laboratory and field conditions. Additionally, a laboratory analysis was made of samples of green sawn lumber for export coming from plantations located in the area of DFP occurrence. The samples were taken in ports from the Bio-Bio Region in order to determine the occurrence of *P. pinifolia*.

The establishment of the occurrence of *P. pinifolia* in samples of green sawn pine lumber, foliage of trees, and bark and wood disks was carried out through cultures in selective medium for species of the genus *Phytophthora*, identification of morphological characteristics (described by Durán and others 2008), and by direct molecular analysis of wood and foliage using *P. pinifolia*-specific primers (developed by Durán and others, in press). The presence of structures of the pathogen was also verified through observations with a fluorescence microscope.

Evaluation of the Occurrence of *P. pinifolia* in Green Sawn Lumber Obtained From Trees Affected by DFP

Selection of Samples

The selection of farms was made based on the historical damage and age of stands. A total of three stands were chosen at Llico, Trana-Trauco, and Quebrada de Rumena Farms. These plantations had damage at least over the last 4 years. In each of these plantations, a total of 15 trees were selected and sampled on the basis of the presence of typical symptoms and signs (black bands on needles or resin tapping on the stem). Prior to harvest, a characterization was made of the DFP degree in foliage, diameter at breast height (DBH), total height, and live crown height, corresponding to the 2008 period. In addition, samples from foliage, and from bark and wood disks were taken to validate the occurrence of *P. pinifolia* in the laboratory.

The number of trees selected was determined according to the standard NCh 1208 (50 percent associated with the average diameter, 25 percent of the upper diameter class, and 25 percent of the lower diameter class). The trees were felled and cut into operational saw logs (3.6 m in length to a minimum diameter of 14 cm) and pulp timber (variable length, diameter 14 to 8 cm). The saw logs were gathered in the forest for 10 days, simulating a harvest operational process of *P. radiata* plantations in Chile.

Sawing Process

Saw logs were transported to a sawmill (Horcones II) yard located in the county of Arauco (Annexe 1), where they were processed. The sawing schemes used were defined depending on the diameter class of the logs, maximizing the obtaining of sideboards in millimetre thickness (side 25 mm and central 38 mm, per variable width).

A total of 40 samples for laboratory analysis were obtained, according to the provisions in the Chilean standard NCh 1208 EOf.76 (rigorous inspection mode). The selection of samples was aimed at logs with visible resin tapping, identified from the forest, with location on the stem (log one, two, three or four) and type of wood (30 percent of samples associated with central wood and 70 percent associated with lateral wood). The samples were characterized to maintain traceability during the whole study process (Annexe 4). The size of the samples obtained were 15 cm long, 25 and 38 mm thick, and variable in width depending on the type of wood obtained after the first 10 cm from one of the ends of the boards.

Once the samples were obtained and sent to the laboratory, the remaining lumber continued with the normal procedure of sawn wood production, being subject to a bath with anti-stain solution made up of a fungicide mixture. After the anti-sapstain treatment, the lumber was stored in the sawmill yard (Aserradero Horcones II).

Laboratory Analysis of Sawn Wood Specimens

The specimens obtained from the sawing process were analyzed through culture and PCR as described below:

Culture: From each specimen obtained, 10 wood pieces were selected, which were cultured in plates with selective medium (CARP). The plates were maintained

between 18 and 22 °C for 30 days until their evaluation. The culture validation was made through molecular biology using *P. pinifolia*-specific primers.
PCR: A chip sample was taken from each specimen. Each sample was evaluated through molecular biology directly from wood, directed to zones with evidence of any kind of stain. The DNA extraction was carried out using CTAB buffer, whereas PCR was made through *P. pinifolia*-specific primers.

Evaluation of the Capability of *P. pinifolia* to Contaminate and Develop on Green Sawn Lumber of *P. radiata*

Specimen Inoculation in the Laboratory

The inoculation of *P. pinifolia* was performed through the application of 250 ul of suspension of 50,000 zoospores per ml on the specimen surface (ASTM D 4445-03, 2003, standard) and with an 8 mm diameter mycelium plug placed in perforations of the same diameter on the specimens. The treatments corresponded to specimens with and without anti-sapstain treatment, whereas the control included specimens inoculated with water and agar plugs without mycelium, respectively (table 1). Inoculum viability was validated using rhododendron bait and later culture of the bait. All treatments were maintained at ± 22 °C in a humidity chamber for 30 days until their evaluation.

Table 1—Treatments of laboratory tests

Treatment Code	Inoculum, Mycelial Plug	Inoculum, Zoospore Suspension	Anti Sapstain Treatment	No. Specimens
T0	No		Yes	10
T1	No		No	10
T2	Yes		Yes	10
T3	Yes		No	10
T4		No	Yes	10
T5		No	No	10
T6		Yes	Yes	10
T7		Yes	No	10

Every week an evaluation was performed through a visual review during the period in which the specimens were kept in humidity chamber in order to observe the occurrence or development of *P. pinifolia*. At day 30, the specimens were evaluated by culturing, PCR directly from wood, and fluorescence microscopy, as described below:
Culture: From each specimen (80 in total), five pieces were selected and cultured in a plate of selective medium (CARP), for a total of 350 cultures. The plates were incubated between 18 and 22 °C for 30 days until their evaluation.
PCR: A chip sample was taken from each specimen. Each sample was evaluated through molecular biology directly from wood. The DNA extraction was carried out using CTAB buffer (Annexe 4), whereas PCR was made through *P. pinifolia*-specific primers.
Fluorescence Microscopy: A section of the inoculated surface was selected from each sample. The samples were immersed in calcofluor for 10 seconds and observed under a fluorescence microscope (Olympus CX31).

On-Site Specimen Inoculation

A total of 128 specimens of *P. radiata* green wood were placed in a stand on the Llico Farm (plantation 2002) with a high incidence of DFP. Under the canopy (foliage) of this plantation, 96 wood specimens were placed on a tray with trap plants to monitor the occurrence of DFP. Additionally, 12 specimens with and without anti-stain treatment were placed in contact with soil, as well as 20 specimens artificially-inoculated with *P. pinifolia*, tied to *P. radiata* trees, which were also inoculated (table 2).

Table 2—Treatments placed in field

Treatment	Specimen Location	Anti-Sapstain Application	No. Specimens
T0	Tray with trap plants	Yes	48
T1	Tray with trap plants	No	48
T2	Soil in contact with trays	Yes	6
T3	Soil in contact with trays	No	6
T4	Branches inoculated with *P. pinifolia*	Yes	5
T5	Branches inoculated with *P. pinifolia*	No	5
T6	Branches non-inoculated	Yes	5
T7	Branches non-inoculated	No	5

The 128 specimens were maintained in the field for 30 days and later taken to the laboratory for their evaluation. The evaluation of the specimens was conducted with the same methodology described above.

Lumber Sampling at Ports

In order to support the management of Agriculture and Livestock Service (SAG) in monitoring green sawn lumber for export, sampling of specimens was performed in Lirquen and Coronel ports to evaluate the occurrence of *P. pinifolia* during August, October, November, and December 2008.

Twenty lumber packages (17 of green sawn lumber and three of dry sawn lumber) were sampled, obtaining specimens especially from boards with bark. The samples were analyzed in laboratory through operational protocol for molecular biology using *P. pinifolia*-specific primers.

Results and Discussion

Occurrence of *P. pinifolia* in Green Sawn Lumber Obtained from Trees Affected by DFP

The evaluation of specimens of green sawn lumber, conducted through molecular biology directly from wood and isolates in selective medium, did not show the occurrence of *P. pinifolia* (table 3).

Table 3—Evaluation of specimens of green sawn wood

Type of Sample	No. Samples	Analysis		*P. pinifolia* Identification	
		PCR	Culture	PCR	Culture
DFP Farm Specimens	40	80	400	0	0
Control Specimens	20	40	200	0	0

Capability of *P. pinifolia* to Contaminate, Colonize, and Survive on Green Sawn Lumber

Specimen Inoculation in the Laboratory

The inoculated specimens with and without anti-sapstain treatment did not show growth of *P. pinifolia* after incubation for 4 weeks (table 4). In specimens treated with anti-stain bath, no growth from any kind of fungi was observed, whereas in those without bath, structures of staining fungi typically seen in *P. radiata* sawn wood developed. This validates that the incubation conditions of the specimens were proper for the growth of fungi.

Table 4—Evaluation of specimens inoculated in laboratory

Type of Sample	No. Samples	No. Analyzed		*P. pinifolia* Identification	
		PCR	Culture	PCR	Culture
Specimens inoculated with zoospores	20	40	100	0	0
Specimens inoculated with mycelium	20	40	100	0	0
Control specimens	40	80	200	0	0

The presence of sporangia or other reproduction structures that may validate the occurrence of *P. pinifolia* was not detected in the evaluations made through fluorescence microscopy. The molecular analysis through PCR was negative for the presence of *P. pinifolia*.

Specimen Inoculation in the Field

The evaluation of the 128 specimens did not show the occurrence of *P. pinifolia* in culture, PCR directly from wood, or humidity chamber (table 5). In specimens without anti-sapstain treatment, some evidence of staining fungus development were found, which suggests that the conditions under which the specimens were maintained were suitable for the growth of fungi and that the anti-stain bath is effective against that type of agent.

Table 5—Evaluation of specimens in field

Type of Sample	Analysis			*P. pinifolia* Identification	
	No. Samples	PCR	Culture	PCR	Culture
Specimens under canopy	96	192	480	0	0
Specimens on soil	12	24	60	0	0
Specimens of inoculated branches	20	40	100	0	0

Lumber Sampling at Ports

The occurrence of *P. pinifolia* was not detected in the evaluations performed on samples obtained at the ports of Lirquén and Coronel from August to December.

Background information to determine contamination of wood by *Phytophthora* species genetically related to *P. pinifolia* was not available; however, other species such as *P. ramorum, P. nemorosa, P.pseudosyringae, P. kernoviae* are able to cause damage on the xylem and produce cankers (Wickland and others 2008, Brown and Brasier 2007).

Conclusions

The results obtained in this study allow us to conclude that:
1. *P. pinifolia* is not present in samples of green sawn lumber obtained from plantations with high incidence of symptoms and damages caused by DFP over the last 4 years, even though the sample selection was directed to conditions more favorable for the presence of the pathogen.
2. When exposed under the canopy of a plantation highly affected by DFP, *P. pinifolia* is not able to colonize and survive on green sawn wood artificially or naturally inoculated with zoospores or mycelium.
3. Green sawn lumber produced from plantations of *P. radiata* infested with *P. pinifolia* are free of the pathogen and may be exported without needing to use treatments against *P. pinifolia*.

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GSOB ≠ SOD: Tree Mortality from the Goldspotted Oak Borer in Oak Woodlands of Southern California[1]

Tom W. Coleman[2] and Steven J. Seybold[3]

Abstract

A new threat to oaks (*Quercus* spp.) in California was identified in June 2008 following years of misdiagnosis. The goldspotted oak borer (GSOB), *Agrilus coxalis auroguttatus* Schaeffer (Coleoptera: Buprestidae), is aggressively attacking and killing three species of oaks in oak woodlands in San Diego County. About 20,000 coast live oaks (*Quercus agrifolia*), California black oaks (*Q. kelloggii*), and canyon live oaks (*Q. chrysolepis*) have died in a 4903 km^2 area centered on the Descanso Ranger District, Cleveland National Forest and Cuyamaca Rancho State Park. Oak mortality has been continuous for the past 8 years and occurs on all land ownerships.

The goldspotted oak borer was first collected in California in 2004. Although the collection history for this species is very limited (68 pinned specimens or records from 26 museum and private collections surveyed), early records date back to 1889 and 1905 in southern Mexico and southeastern Arizona, respectively, where there have been no reports of damage or mortality to oaks. In fact, prior to 2008, the biology and hosts of GSOB were unknown. Previous collection history for GSOB, the pattern of oak mortality in California since 2002, an expanding level of infestation, and geographical separation of oak stands in southeastern Arizona and southern California strongly suggest that GSOB was introduced into San Diego County. Although molecular genetic analyses of the populations of GSOB are pending, the proximity of Arizona and California and the morphological similarity of specimens from the Arizona and California populations both imply that GSOB was introduced into San Diego County from Arizona. Firewood movement represents the most likely pathway into California, however, in support of an alternative hypothesis of origin, there are anecdotal reports of oak firewood brought into this area of San Diego County from Mexico for 20 years.

Larval GSOB kill native oaks by feeding primarily on the wood surface at the interface of the xylem and phloem. Larvae feed in a meandering pattern on the wood surface and galleries typically have a dark appearance when bark is first removed. Larval feeding can reach high densities and cause areas of the cambium to die, eventually leading to tree mortality. Oaks infested with GSOB can be identified by thinning crowns, D-shaped adult exit holes, woodpecker foraging, and dark black or red staining on the bole or larger branches. Evidence of colonization by other insects is absent in GSOB-infested trees until trees have declined severely (for example, when bark has cracked around areas of dead cambium). Studies are currently underway to assess GSOB biology, enhance survey and monitoring techniques, determine the distribution of GSOB in southern California, record its impact to native oak stands, manage its populations through insecticide and firewood treatments, and determine

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whether there is a relationship between physiological stress of oaks and mortality by GSOB. Data on the physiological host range, freezing tolerance, optimal developmental temperatures, and dispersal capacity are being gathered to utilize in a risk assessment.

We hypothesize that elevated oak mortality in southern California has occurred because of an absence of evolved host resistance in native oaks and/or an absence of natural enemies found in GSOB's native range. Observations in California suggest that GSOB tends to prefer coast live oak and California black oak more than canyon live oak; Engelmann oak (*Q. engelmannii*) has not been found with GSOB injury. Preliminary observations from Arizona and California suggest that GSOB may confine its attacks to red oak species (subgenus *Quercus*, section *Lobatae*). We hypothesize that phloem thickness, bark structure, and host chemistry may influence susceptibility to GSOB. The native distributions of the three California hosts of GSOB extend north through most of the state along the coastal foothills and along the Sierra Nevada. Thus, this new pest to oaks has the potential to impact more northern regions in California, and the prospect of GSOB and SOD acting simultaneously on coast live oak in California presents a severe threat to this key oak species.

Introduction

Since 2002, aerial survey data have revealed extensive oak mortality on federal, state, tribal, and private lands in San Diego County, California. About 20,000 coast live oaks (*Quercus agrifolia*), California black oaks (*Q. kelloggii*), and canyon live oaks (*Q. chrysolepis*) have died in a 4903 km^2 area centered on the Descanso Ranger District, Cleveland National Forest and Cuyamaca Rancho State Park. Drought was considered the principal cause of this tree mortality for many years, and various pathogens have been suspected, but never confirmed. In 2008, the goldspotted oak borer (GSOB), *Agrilus coxalis auroguttatus* Schaeffer (Coleoptera: Buprestidae), was linked to the continuing oak mortality (Coleman and Seybold 2008a).

Goldspotted oak borer larvae feed primarily at the interface of the phloem and xylem (Coleman and Seybold 2008b). Larval galleries are meandering and dark-colored. Pupation occurs in the outer bark. Infested trees can be identified by woodpecker foraging on the outer bark, crown thinning and die back, D-shaped adult emergence holes, and dark-colored staining on the bark. Bark staining signifies extensive injury from larval feeding, which eventually girdles trees and leads to their death. GSOB attacks oaks aggressively along the main stem and largest branches (>12 cm in diameter). No additional insect species are associated with early GSOB injury.

The phloem/wood borer was first collected in 2004 in California. An investigation of the collection history of GSOB (68 specimens or records from 26 collections) revealed that it was first recorded in the 1880s in Guatemala and southern Mexico, and then later in the early 1900s in southeastern Arizona, suggesting that GSOB is native to Central and North America (Coleman and Seybold 2009). No tree injury or mortality has ever been reported from GSOB in these native regions. Very little data on host of development, biology, and life cycle of GSOB were available prior to 2008.

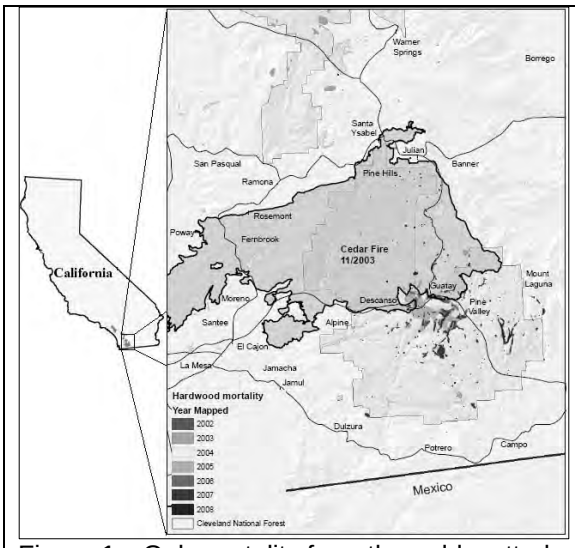

Figure 1—Oak mortality from the goldspotted oak borer in San Diego County, 2002 to 2008.

We hypothesize that GSOB arrived in southern California during the last 10 to 15 years as a consequence of an introduction on oak firewood. Oak mortality was initially detected on the Descanso Ranger District, Cleveland National Forest and at Cuyamaca Rancho State Park, so the GSOB infestation is believed to have originated in the vicinity of these two areas. In 2003, the majority of oak mortality was primarily surrounding the communities of Descanso and Guatay. In the following years (2004 to 2008), oak mortality has expanded outward from these communities (Fig. 1). As of summer 2009, mortality was not present at the U.S. and Mexican border (for example, the town of Campo), and GSOB infested trees along the border were first detected in 2009 and infestation rates are currently very low, suggesting a nascent infestation in this area that is spreading from the north. Increased levels of oak mortality have not been aerially mapped further south of the U.S. and Mexican border, but the spatial pattern of infestation on the U.S. side does not support a range expansion from Mexico. Confirmed oak hosts for GSOB in California have limited distributions in northern Baja California, Mexico and do not extend much further east in San Diego County. Thus, a continuous range expansion of GSOB from Arizona and/or Mexico is extremely unlikely. The Sonoran and Mohave Deserts geographically isolate the oak hosts and the California GSOB population from native GSOB populations in southeastern Arizona and Mexico. Because the zone of mortality is isolated by the Sonora and Mohave deserts to the east and by a band of healthy host type to the south and southeast, and because initial mortality was mapped away from the border, we conclude that the hypothesis of continuous range expansion is unlikely. The recent designation of subspecific status of the California population (Hespenheide and Bellamy 2009) based on its morphological similarity to the Arizona population, suggests that the tree-killing population of GSOB in California originated from Arizona.

Studies in 2009 and 2010 are assessing the current distribution of GSOB in southern California, determining effective survey techniques, investigating the impact of GSOB on forest stand dynamics, documenting adult emergence from firewood, developing management techniques for firewood, exploring the role of oak volatiles in attraction, assessing its risk to areas outside of its current distribution, and investigating the relationship between tree water stress and successful attack. This presentation compares the introduced GSOB population in California with native GSOB populations in Arizona.

Methods and Results

In February 2009, we surveyed two mountain ranges, the Huachuca and Santa Rita Mountains, on the Coronado National Forest in southeastern Arizona (Cochise, Pima, and Santa Cruz Counties) for the presence of GSOB. Early collection records had documented several canyons in these mountains where GSOB had occurred. Aerially detected hardwood mortality is not available for this region, so comparison of long-term oak mortality between Arizona and California is not possible, but our observations suggested that oak mortality was occurring at latent levels in Arizona. The native oak component in these two ranges is primarily comprised of silverleaf oak (*Q. hypoleucoides*), Emory oak (*Q. emoryi*), Arizona white oak (*Q. arizonica*), gray oak (*Q. grisea*), and gamble oak, (*Q. gambelii*). None of these oaks occur in southern California. In Arizona, silverleaf, Emory, Arizona white, and gray oaks are found frequently interspersed in the same forest stand, whereas gamble oak is primarily restricted to higher elevations and was not observed during our survey.

During the detection survey, we observed D-shaped exit holes, meandering dark-colored larval galleries on the sapwood, and pupal cells in the outer bark of several silverleaf oaks and Emory oaks in canyons where previous GSOB specimens had been collected, as well as in new localities. Injury symptoms were similar to those discussed in Coleman and Seybold (2008). Callus tissue was also observed in areas where insect herbivory was present. Mature *Agrlius* sp. larvae were collected from the outer bark of an Emory oak that recently died at one location (Box Canyon) in the Santa Rita Mountains (Pima County) in February 2009. No visible external injury or signs of pathogen infection to the Emory oak were observed. It is believed the tree succumbed due to insect infestation, but predisposing factors may have been involved. The larvae from this original collection did not complete development, so we could not verify the species status of the adult, but we suspected that it was GSOB. The same tree was visited on 1 to 2 May 2009, at which time additional *Agrilus* larvae and pupae were collected. The outer bark of the Emory oak (47 cm diameter at breast height) from ~1.5 m and below to the root collar was removed, collected, and returned to the lab to monitor insect emergence. Bark samples were placed in emergence cages and monitored daily.

The goldspotted oak borer was the only phloem/wood borer that emerged from the bark samples. A total of 113 insects were reared from the bark samples, 102 GSOB, eight parasitoid wasps (Hymenoptera), one moth (Lepidoptera), and two other beetles (Coleoptera). Larvae of one parasitoid species were observed feeding on GSOB larvae in the bark. Of the 27 GSOB larvae and pupae encountered in the bark, four were parasitized (15 percent parasitism rate).

Discussion

Our preliminary observations in California suggest that GSOB tends to prefer coast live oak and California black oak (both “red” oaks, subgenus *Quercus*, section *Lobatae*) more than canyon live oak (an intermediate oak species, i.e., neither a red nor a white oak, subgenus *Quercus*, section *Quercus*) (Nixon 1993). There have been no observations of injury by GSOB to Engelmann oak (*Q. engelmannii*), a white oak species that occurs in San Diego County. During surveys in the Huachuca and Santa Rita Mountains of southeastern Arizona (Cochise, Pima, and Santa Cruz

Counties), we observed similar injury symptoms of GSOB on silverleaf oak and Emory oak (both thick-barked red oaks). On one particular Emory oak, we noted the characteristic injury symptoms and we reared GSOB adults from the outer bark collected at a location in the Santa Rita Mountains (Pima County). This is the first confirmed record of a host for GSOB in Arizona. We suspect that silverleaf oak is also a susceptible host to GSOB because of the injury symptoms that we observed. We found no evidence that the two native Arizona white oaks, Arizona white oak and gray oak, had injury symptoms from GSOB or any woodboring flatheaded borers (Buprestidae). We hypothesize that phloem thickness, bark structure, and host chemistry may influence susceptibility to GSOB. White oaks commonly have fibrous, furrowed bark and thin phloem, whereas red oaks have thick phloem. Additional observations and experiments involving host susceptibility are needed to test this hypothesis.

The native distributions of the three California hosts of GSOB extend north through most of the state along the coastal foothills and along the Sierra Nevada (Coleman and Seybold 2009). The buprestid is currently injuring and killing three oak species between 90 and 1830 m elevation in southern California. Previous collection records in its native region extend to 2195 m. Thus, this new pest to oaks has the potential to impact more northern regions in California, and the sobering possibility of GSOB and SOD impacting coast live oak in unison in the central and southern Coast Range is very worrisome for the future of this beloved California oak. Firewood movement represents a significant pathway for introducing GSOB into these regions and should be a major focus of management efforts in the state.

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Nurseries

A Systems Approach for Detecting Sources of *Phytophthora* Contamination in Nurseries[1]

Jennifer L. Parke,[2] Niklaus Grünwald,[3] Carrie Lewis,[2] and Val Fieland[4]

Introduction

Nursery plants are susceptible to several diseases caused by *Phytophthora* species. Nursery plants are also important long-distance vectors of non-indigenous pathogens such as *P. ramorum* and *P. kernoviae.* Pre-shipment inspections have not been adequate to ensure that shipped plants are free from *Phytophthora*, nor has this method informed growers about sources of contamination in their nurseries.

We applied an approach based on Hazard Analysis of Critical Control Points (HACCP) for systematically detecting sources of *Phytophthora* contamination in four Oregon nurseries. HACCP is an approach broadly applied in the food processing industry to prevent contamination of foods by microorganisms and it has recently been adapted by the U.S. Fish and Wildlife Service to prevent spread of non-target species during fish restocking efforts. Our goal was to adapt the HACCP approach to identify critical control points (CCPs) for *Phytophthora* contamination in commercial nursery production systems. Critical control points are the best points, steps, or procedures at which significant hazards of contamination can be prevented or reduced to minimum hazard. To our knowledge, this is the first time the approach has been used to detect sources of plant pathogens.

Methods

We sampled four Oregon nurseries every 2 months over a 3-year period. The nurseries, designated as A, B, C, and D, ranged in size from 70 to 2200 acres. Two of the nurseries (A and C) used recycled irrigation water, whereas nurseries B and D used well water. Plants, potting media, containers, irrigation water, greenhouse soil, and can yard substrates were sampled at all stages of production. We initially focused on four genera of *Phytophthora*-susceptible plants commonly grown in Oregon: *Rhododendron, Pieris, Kalmia,* and *Viburnum*. Later, the study expanded to include additional host plant genera. If symptomatic plants were available, leaves, stems, and roots of symptomatic plants were plated onto the selective medium PARBH; otherwise asymptomatic tissue was plated. Samples from irrigation water and

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irrigation ponds, soil/gravel substrates, potting media and potting medium components, and debris from used containers were baited in a rhododendron leaf assay and plated onto the selective medium. Pure cultures were isolated and putative *Phytophthoras* were identified to species by direct sequencing of the internal transcribed spacer (ITS) rDNA and blast searches at www.phytophthora-id.org.

Results

Critical control points in the nurseries were 1) placement of healthy container-grown plants on contaminated soil/gravel substrates (all nurseries), 2) use of contaminated irrigation water (nurseries A and C only), and 3) use of contaminated used pots (all nurseries). Poor sanitation practices were compounded by the accumulation of standing water in parts of all nurseries. After identifying CCPs where contamination occurred, we worked with nursery managers to develop best management practices specific for each nursery.

Of 449 total *Phytophthora* isolates recovered, 364 isolates (81 percent) belonged to 15 *Phytophthora* species, 13 percent matched *Phytophthora* taxa without species designations, and 6 percent did not match any sequence in the *Phytophthora* database. The most frequently isolated species from symptomatic plants were *P. citricola*, *P. cinnamomi*, *P. citrophthora*, and *P. syringae*. From gravel substrates, pots, and soil, the predominant species were *P. citricola*, *P. cinnamomi*, and *P. cryptogea*. From irrigation ponds, most isolates were *P. gonapodyides* or other *Phytophthora* taxa belonging to ITS Clade 6. *Phytophthora parsiana*, not previously reported from nurseries, was also detected. *Phytophthora cinnamomi*, one of the species isolated most frequently from plants, was never recovered from water.

Discussion

Identification of critical control points is essential for determining specific sources of *Phytophthora* contamination in nurseries and designing effective management strategies to prevent disease. The systems approach could be applied to other pathogens and pests to ensure the health of nursery stock. Our results also provide insights on *Phytophthora* and ecology and epidemiology in nurseries.

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Detection of *Phytophthora ramorum* at Retail Nurseries in the Southeastern United States[1]

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Abstract

Many nursery plants are known to be hosts of *Phytophthora ramorum* or to be associated with this pathogen. These plants can be infected or merely infested by *P. ramorum* and with or without symptoms. The pathogen has been detected most frequently on container-grown nursery plants, and occasionally has been found in the container mix around these plants. Because of this close association with container-grown nursery plants, *P. ramorum* can be disseminated within a nursery, among local nurseries, and between nurseries in different states on infected plants or infested materials. Pathogen propagules can be moved readily from nurseries or landscapes to natural ecosystems in soil and water. Therefore, a major concern is that *P. ramorum* will be introduced into nurseries in the eastern United States (U.S.) and then will become established in forests and other natural areas where environmental conditions are favorable for pathogen survival and reproduction and susceptible plants are present. Consequently, we have been cooperating with the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS) to monitor for *P. ramorum* in soil and water at retail nurseries in the southeastern U.S. when infected plants have been found by routine surveys.

To detect *P. ramorum* in field soil and container mix, a representative composite sample of at least 1 liter was obtained by collecting and combining multiple subsamples (100 to 200 ml each). Subsamples of field soil (composed of soil, gravel, peat fines, and other substrates) were collected to a depth of 10 to 20 cm around and under container-grown plants known or suspected to harbor the pathogen and along the drainage path of runoff water leaving an area of suspect plants. Subsamples of container mix were collected from all depths in pots containing plants with or without typical symptoms of *Phytophthora* foliage blight—usually with one subsample taken from each plant in a block. In the laboratory, each soil sample was mixed thoroughly and tested for *Phytophthora* spp. with a baiting bioassay. Three subsamples were removed from each composite sample and flooded with distilled water. Small pieces of camellia and rhododendron leaves (5 × 5 mm) were floated on the water, and baited soils were held at 20 °C for 3 days. Baits were removed, rinsed in distilled water, blotted dry, and embedded in PARPH-V8 selective medium. Isolation plates were held at 20 °C for up to 4 weeks and were examined regularly for colonies of *P. ramorum*.

To detect *P. ramorum* in water, samples (1 or 2 liters in volume) were collected in 100 ml aliquots from irrigation runoff water in ponds, streams, retention basins, drainage ditches, and puddles on nursery property. Samples were assayed by filtration within 6 hours of collection. For each sample, eight or nine aliquots (50 to 200 ml each) were passed through membrane filters with 3 or 5 µm pores. Filters were inverted onto PARPH-V8 selective medium, and

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plates were held at approximately 20 °C in the dark for 2 to 3 days. Filters then were removed and colonies of *Phytophthora* spp. were counted; representative colonies were subcultured and saved for identification. Plates were examined for colonies of *P. ramorum* for up to 21 days.

Table 1—Occurrence of *P. ramorum* at five garden centers and nurseries in three states from 2006 through early 2009: on plants, in irrigation runoff water, and in soil associated with diseased plants

Site	Date		*P. ramorum*+ plants[a]	Irrigation runoff[b]		"Soil"[c]	
	Year	Month		Pond	Ditch	Pots	Field
FL: Retail garden center	2006	Feb	camellia	-	+		+
		Mar		-	-		-
	2007	Mar	camellia	+	-		+
		Apr		-	-		+
		Dec		-		-	-
	2008	Feb	viburnum, loropetalum	-	-	+	+
FL: Production nursery	2008	Feb			+		+
	2009	Mar			-		-
MS: Retail garden center	2008	Feb	magnolia		+		+
		Apr 01	magnolia		+		
		Apr 29	??		-		
	2009	Jan			+		-
		May			-	-	-
SC: Retail garden center	2008	Jun	kalmia				
		Jul	azalea, pieris		-	+	+
	2009	May	pieris			-	+
SC: Retail nursery	2008	Nov	rhododendron				
		Dec	rhododendron		-	+	+
	2009	Jan	kalmia, pieris		-	-	+
		Feb				-	
		May	rhododendron			-	+

[a] Plants infected by *P. ramorum* usually were diagnosed based on samples sent to state or federal laboratories.

[b] Irrigation runoff water was sampled in a holding pond at one Florida location and in drainage ditches.

[c] Soil samples were collected from field soil under diseased container-grown plants and from the container mix in the pots containing diseased plants.

Five nurseries in three states have been sampled repeatedly between 2006 and early 2009 (table 1). We also have detected *P. ramorum* at other retail nurseries and garden centers in Alabama, Georgia, and North Carolina. *P. ramorum* continues to occur on container-grown plants in retail nurseries in the southeastern U.S. The source of infection on these plants was not always clear; however, evidence suggests two scenarios: (1) plants already were infected before arriving at the nursery or garden center or (2) plants became infected after arriving at the nursery or garden center. Plants that were infected before arriving at nurseries and garden centers did not come from nurseries only in California, Oregon, and Washington, where *P. ramorum* is most common. This pathogen has escaped from diseased plants into field soil and has become established at some of these locations—where resident inoculum appears to have caused new infections on otherwise healthy plants. *P. ramorum* also is moving off-site in runoff water at some nurseries and garden centers. At several locations, runoff water was headed to forested ecosystems and local waterways. It is likely that infected or infested plants

have been sold and may have been planted in residential landscapes. Because *P. ramorum* has been found only where intensive surveys have been conducted, it is very likely that it is present at nurseries and garden centers in other southeastern states. Now it must be determined if *P. ramorum* has moved into the natural environment in the southeastern U.S. The sampling efforts at each of the five nurseries and garden centers listed in table 1 are summarized below.

Florida. From 2006 to 2008, a retail garden center in northern Florida was surveyed six times for *P. ramorum*. In February 2006, *P. ramorum* was found in runoff water in a retention basin and a connecting drainage ditch and in field soil. The ditch carried runoff water off-site to a wooded natural area. Infected camellias with sporulating lesions were present at the nursery; these plants had been received recently from an out-of-state nursery. *P. ramorum* was not detected in samples collected 1 month later. Infected camellias received from an out-of-state nursery again were present in early 2007 (prior to sampling in March) but were moved inadvertently to an off-site production nursery operated by the garden center. In samples collected in March 2007, *P. ramorum* was detected in field soil where diseased plants had been placed and in a pond where runoff water collected prior to leaving the garden center. The pathogen was detected again in a field soil sample collected 1 month later, but all samples collected in December 2007 were negative. In February 2008, viburnum and loropetalum plants at the garden center were found to be infected by *P. ramorum*. It also was detected in the field soil under and around these plants as well as in the container mix in the pots. It appeared that the inoculum initiating infections on these plants was being splashed up from the infested soil. During a visit to the production nursery where infected camellias from the garden center had been moved in 2007, *P. ramorum* was detected in field soil where the camellias had been located and in runoff water draining this location, which was moving away from the nursery into a forested area.

Mississippi. A retail garden center in Mississippi was surveyed five times, three times in 2008 and twice in 2009. Although thorough sampling in the garden center was not permitted until May 2009, a few soil samples were collected by state personnel and shipped to us in 2008 and early 2009. *P. ramorum* was detected in a field soil sample collected around the edge of an asphalt-paved pad where infected magnolia plants were located in February 2008, and it was repeatedly detected in runoff water leaving the nursery and heading for a local creek, which meandered through a forested area.

South Carolina. A retail garden center and a separate retail nursery in South Carolina were surveyed several times in 2008 and 2009. Infected kalmia plants were found at the garden center in June 2008. In July, *P. ramorum* was isolated from azalea and pieris plants, container mix in these pots, and field soil from around these plants, but it was not detected in water within and immediately outside the nursery. Infected pieris plants that were not present the previous summer were found in May 2009 during a follow-up survey, and the pathogen was recovered in a field soil sample from an adjacent block of plants. At the second location, the retail nursery was found to have diseased rhododendrons in November 2008, and *P. ramorum* was detected in soil samples collected in December. However, it was not detected in container mix samples collected from additional plants at this nursery in February 2009. *P. ramorum* was detected again in May 2009 on rhododendron plants that had been received recently from out-of-state nurseries. It was present in field soil under and around these plants but was not detected in the container mix from these pots. Most of the symptoms on the diseased plants were on lower branches, which suggested that the inoculum was resident in and splashing up from the field soil around these plants.

Within-Field Spread of *Phytophthora ramorum* on Rhododendron in Nursery Settings[1]

Kurt Heungens,[2] Isabelle De Dobbelaere,[2] Bjorn Gehesquière,[2] Annelies Vercauteren,[2] and Martine Maes[2]

Abstract

In Europe, *Phytophthora ramorum* has mostly been detected on rhododendron plants in nurseries. European Union (EU) phytosanitary measures state that potential host plants within a radius of 2 m of an infected plant must be destroyed, and remaining host plants within a radius of 10 m must be quarantined. Despite the lack of research on the spread characteristics of *P. ramorum* in nurseries, these distances have been generally accepted.

To test the distances over which *P. ramorum* can spread between potted rhododendron plants in a nursery setting, dispersal was monitored from individual infected plants in a mock nursery. The distance over which the disease could spread was monitored in separate experiments during spring, summer, and fall seasons. Dispersal of the pathogen was mainly to neighboring plants, and plant-to-plant contact was an important factor for successful spread. Aerial detection of *P. ramorum* with a Burkard spore sampler was consistently negative. In contrast, pathogen spread via drain water films on the non-draining growing surface took place even at a distance of several meters. Splash dispersal from water films and direct inoculation during tipping over of plants seem to play a more important role in pathogen spread than aerial dispersal.

Disease spread was also monitored during the course of 2 seasons at a commercial nursery where a large number of findings of *P. ramorum* had been made during the EU-mandated survey. Plant parts with *Phytophthora*-like symptoms were collected at regular intervals, field coordinates of samples were taken, and the pathogen was isolated and identified. Out of more than 1300 samples, *P. citricola* was the most abundant *Phytophthora* species present, but a total of 281 *P. ramorum*-positive samples were identified. The location of *P. ramorum* findings was correlated with cultivar block within the field and did not show a clear pattern of focal spread. Microsatellite-based genotyping of the *P. ramorum* isolates revealed two sets of isolates with a unique genotype. Distance in the field between isolates of the same genotype was not limited to 10 m. Presence of *P. ramorum* in drain water was confirmed.

These data again suggest that aerial dispersal of *P. ramorum* is limited, but spread via drainage water can occur over several meters on the non-draining surfaces, and is the most likely means of spread among potted plants grown under such conditions. These data have implications for both quarantine measures and practical pest management.

Introduction

In Europe, rhododendron is the main host plant for *Phytophthora ramorum*. European

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Union (EU) phytosanitary measures for nurseries include destruction of all host plants within a 2 m radius of an infected plant, while remaining host plants within a 10 m radius are quarantined. These distances have not yet been backed by data on in-field spread of *P. ramorum*, despite the need for measures that are both appropriate for successful pest management as well as sensitive to economic damage incurred by the grower. Studies of plant-to-plant spread in nursery settings have been limited. Tjösvold and others (2006) observed no spread beyond 30 to 50 cm, but the leaf area of the detector plants in their study was limited. Neubauer and others (2006) were not successful in observing spread of *P. ramorum* in a nursery experiment due to a rapidly spreading *P. citricola* co-infection. Werres and others (2007) observed spread from irrigation water, but their study did not focus on plant-to-plant spread. In light of the dearth of information on plant-to-plant spread in nurseries, the objective of this study was to directly and indirectly monitor the distance of plant-to-plant spread of *P. ramorum* in (mock) nursery settings and to determine the relative importance of aerial versus water-film dispersal.

Materials and Methods

At a mock nursery where appropriate biosafety measures were taken, pathogen dispersal was monitored from a centrally placed *P. ramorum*-infected rhododendron plant (cv. Mme Masson) to up to three concentric circles of detector plants. Three to four replicate sets were installed. Frequent overhead irrigation events lead to conditions conducive for sporulation and infection. After 7 days, plants were labelled, covered with a plastic bag, and placed at 17 to 20 °C for an additional week, after which symptoms and presence of *P. ramorum* was determined. Separate experiments were conducted during spring, summer, and fall seasons. Two similar experiments (in fall and spring) were conducted with single circles of detector plants, placed either in contact, or at distances of 5 or 30 cm from the infected plant. During these experiments, aerial detection of *P. ramorum* was monitored with a Burkard spore sampler that was placed just outside the outer circle of the detector plants. Detection of *P. ramorum* in the run-off water was performed via leaf baits or via direct molecular detection (real-time PCR). In a separate experiment, a Burkard spore sampler was placed next to and downwind from infected plants in order to aerially detect *P. ramorum* spores. To evaluate infection of plants that have tipped over in a water film containing *P. ramorum*, plants were immersed for 10 seconds 1 hour, 2 hours, or 4 hours in a zoospore suspension and symptom development was then monitored.

Presence and spread of *P. ramorum* was also monitored at a production nursery during the 2004 and 2005 growing seasons. From June to October, at 4 to 6 week intervals, all twigs and leaves with *Phytophthora*-like symptoms were collected, field coordinates of samples were taken, and the pathogen was isolated and identified. Isolates of *P. ramorum* were genotyped with EU1 polymorphic microsatellites according to Vercauteren and others (2010). Drain water was monitored for *P. ramorum* presence as described above.

Results and Discussion

At the mock nursery, dispersal of the pathogen from the centrally-placed infected plant to the detector plants that were placed in surrounding concentric circles was

mostly limited to the first circle of plants (78, 38, and 75 percent disease incidence in the three trials). Occasional spread to the second circle (28, 2, and 8 percent disease incidence in the three trials) and third circle (0 and 7 percent disease incidence in the last two trials) was observed. The level of disease incidence was correlated with the conduciveness of the weather conditions during the given test. Detector plants in the first circle invariably showed most symptoms at the side of the plant that was in direct contact with the detector plant.

The dispersal of the pathogen to the single circle of detector plants, placed either in contact with or at distances of 5 or 30 cm from the infected plant, clearly showed that pathogen spread required direct contact between the plants. Plants in direct contact showed symptoms (25 and 69 percent disease incidence in the two trials) while plants at 5 cm only showed symptoms if contact due to wind was not prevented (6 percent disease incidence in both trials without contact prevention, 0 percent if contact prevention via wire mesh), and plants at 30 cm did not show any symptoms. Aerial detection of *P. ramorum* with a Burkard spore sampler was consistently negative, even when the sampler was placed immediately adjacent to infected plants. In contrast, pathogen detection in drain water films and run-off water was consistently positive, even at several meters from the infected plants. Tipping over of plants in zoospore-containing suspensions for 1 hour or more resulted in heavy disease symptoms. However, lighter symptoms were already present after a 10 second zoospore dip. Taken together, these experiments indicate that aerial dispersal is very limited and above-ground plant-to-plant pathogen transfer requires direct contact. Similar evidence for short distance aerial dispersal in a mock nursery setting was observed by Tjösvold and others (2006). In contrast to short distance aerial dispersal, splash dispersal from water films (on non-infiltrating growing surfaces) and direct inoculation during tipping over of plants seem to play an important role in pathogen spread.

Over 1300 samples were taken during the surveys at the commercial production nursery. Approximately 16 percent of the samples were positive for *P. ramorum*, while *P. citricola* was the dominant species (> 70 percent). Dominance of *P. citricola* was also observed in the dispersal experiments of Neubauer and others (2006). Distribution of *P. ramorum* was not linked to clear disease foci, although the incidence was higher in specific cultivar blocks and was probably linked to the susceptibility of the given cultivars. No clear spread pattern was observed. Microsatellite-based genotyping of the *P. ramorum* isolates revealed several individual genotypes, as well as two sets of isolates with a unique genotype. Distance in the field between isolates of the same genotype was not limited to 10 m, indicating that spread of the pathogen in commercial settings is not necessarily limited to the 10 m diameter quarantine area. Presence of *P. ramorum* in drain water was confirmed at the commercial nursery. These results, together with those from the mock nursery experiments, suggest that spread at the commercial nursery was most likely the result of water-film mediated dispersal of the pathogen.

These data suggest that containment measures in these types of nurseries should not focus on prevention of aerial spread, but rather prevention of water-mediated spread. Zoospores in water films can infect above-ground plant parts via splash dispersal. They may also be able to infect roots, where they can reside in a latent state. Tipped-over plants should be destroyed, or at least separated and observed.

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Importance of Rainfall and Sprinkler Irrigation in Supporting Sporulation, Spread of Inoculum in Runoff-Water, and New Infections of *Phytophthora ramorum* Under Field Conditions[1]

Steve Tjosvold,[2] David Chambers,[2] and Elizabeth Fichtner[3]

Abstract

If a nursery plant infected with *Phytophthora ramorum* is introduced in a non-infested area, then it is important to understand what environmental conditions could lead spread and infection of new hosts. Once an infected nursery plant is introduced in a nursery or landscape, moving water sources, such as from rain and irrigation events, could provide an important means for dispersal and infection of new hosts. We evaluated seasonal environmental factors and associated rainfall and irrigation events that could support sporulation, dispersal, and infection of new hosts under field conditions.

Ten leaves on each rhododendron and camellia in a 3.8 l pot were inoculated with *P. ramorum* every 3 months (once each season) from July 2006 to June 2008 and grown in field conditions. The pots of each species were grouped into six blocks with seven pots per block. Approximately every 2 weeks for 3 months after each seasonal inoculation, a 1.2 m by 1.2 m tray was placed under each block to collect the majority of run-off water that fell through and around the plant's foliage during a rainfall event. A smaller portion of run-off water was allowed to run down a small opening around the base of the plant and infiltrate into the potting soil. If there was no rainfall during the period, then the pots were sprinkler irrigated for 45 minutes in early morning. The run-off's inoculum concentration was assessed by filtering with a Milipore filter and plating the filters on PARP semi-selective media and counting *P. ramorum* colonies for up to 3 months after inoculation. At the end of the rainfall or irrigation event, 10 infected leaves from each block were selected and each leaf was washed on both sides with a strong stream of 100 ml distilled water. The concentration of the leaf wash was quantified by plating 1 ml subsamples directly onto a 10 cm Petri plate with PARP semi-selective media and counting *P. ramorum* colonies for up to 6 months after inoculation. The soil leachate, coming from the bottom of each pot, was collected in plastic bags and the inoculum concentration was assessed with pear baits for up to 3 months after inoculation. For each species, each of three 1 l subsamples of leachate was baited with one unripe pear, and resulting lesions on the pears were counted.

The disease risk was assessed in the field or in the laboratory. For the field assessment, the run-off water was collected from each species and sprayed onto three plants of the respective species. The plants were incubated in plastic covered tubs in shaded field conditions for 48 hours, removed from the tub, and periodically inspected for symptomatic leaf lesions. Beginning with the winter season of the first year, six 8 mm-diameter leaf disks of each

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species were flooded in three Petri plates containing 6 ml run-off water from each respective species. The leaf disks were periodically inspected for characteristic lesions, and infected leaf disks were counted. Six camellias inoculated in fall 2006 were evaluated for root infections about 6 months after inoculation. The number of consecutive hours that leaves were wet preceding the run-off or leaf wash sampling was measured by a leaf wetness sensor in the plant leaf canopy.

Although the highest concentrations of inoculum were found in fall (October to December) and winter (January to March) of both years, inoculum could be found in all seasons in run-off water up to the last evaluation (3 months after inoculation) and in leaf washes up to the last evaluation (6 months after inoculation) for each inoculation period. High concentrations of inoculum in run-off water and leaf washes were more commonly found after rainfall events than after irrigation events, and the concentration was positively correlated to the number of consecutive hours of leaf wetness. In general, inoculum concentration in the soil leachate was correlated with the volume and inoculum concentration of the water run-off indicating that inoculum ran through the potting soil relatively easily. However, there were also periods when the concentration of soil leachate did not significantly increase until after several weeks of the initial inoculation even though high inoculum was found in run-off water and leaf washes. Four of the six camellia pots sampled about 6 months after inoculation had roots infected with *P. ramorum*, and inoculum was detected in soil leachate even though the plants were completely defoliated after 3 months. The inoculum in the soil leachate may have been produced by sporulating infected roots. Disease on plants sprayed with run-off water occurred only in the winter of the first year after an extensive cool and rainy period. Leaf disk infection was a better measure of disease risk. Leaf disk infection was positively correlated with the concentration of inoculum in the run-off water, and leaf disk infection occurred only in the wet periods of the second year.

Rainfall events create the conditions for extended leaf wetness needed for sporulation of leaf lesions. In our study, irrigation events did not usually extend overnight leaf wetness; however, we noted that spring and fall conditions with clear cold nights could create heavy overnight dew, and leaf wetness could be extended by early morning irrigation. If dew exists in the morning, nursery operators should delay any necessary irrigation until the leaves can dry and the wet period is broken. Run-off water coming off of sporulating plants could contain sufficient inoculum to cause disease under favorable environmental conditions. Run-off water could cause secondary leaf infections on the same plant or perhaps after it flows in the nursery drainage and splashed onto other plants. Inoculum in run-off water could be splashed from the drainage onto plants or come into contact with roots and cause root infections. Run-off water that is recycled for use in the nursery could pose a risk if it is not treated with chlorine, ozone, ultra-violet light, or other sanitation treatment.

A Test System to Quantify Inoculum in Runoff from *Phytophthora ramorum*-Infected Plant Roots[1]

Nina Shishkoff[2]

Introduction

Foliar hosts of *Phytophthora ramorum* are often susceptible to root infection, but the epidemiological significance of such infections is unknown. We used a standardized test system to study inoculum in runoff from root-infected *Viburnum tinus* cuttings.

Test System

The test system was designed for use with *Viburnum tinus* as the positive control. Cuttings from this plant root readily at all times of year and are uniform in size when taken from single node/internode stem segments. Cuttings were produced in 32-insert trays filled with Turface® MVP® (a calcined, montmorillonite clay substrate medium manufactured by Profile Products LLC 750 Lake Cook Road, Suite 440 Buffalo Grove, IL 60089) under mist. After plants had rooted, they were inoculated by pouring 15 ml of a sporangial suspension (500 sporangia/ml) over roots, waiting 24 hours, and then transplanting the carefully washed plants into 2 x 2 inch plastic pots containing clean Turface® (fig. 1a). The bottom of each pot was lined with a plastic mesh insert for easy drainage (fig. 1b). To quantify inoculum in runoff from an inoculated plant, a sufficient volume of distilled water was poured into the pot so that 15 to 20 ml of runoff could be collected in a plastic centrifuge tube. Runoff was then subsampled using a plastic syringe to add 1 ml aliquots to three plates of PARPH selective media. Plates were swirled to distribute a film of runoff over the entire surface, and then incubated at 20 ºC. Plates were subsequently examined weekly for 3 weeks and all colonies counted using a dissecting microscope with dark-field illumination (under which colonies of *P. ramorum* are characteristically highly refractive). Runoff samples were taken periodically (every 3 days or weekly), allowing regression analysis for a mixed model (samples over time) to be done. At the end of the experiment, roots were washed or surface-sterilized for 60 seconds and then 1 cm segments plated on PARPH selective media; the remainder of the root system was dried at 100 ºC. Ideally, 120 root segments total were plated, fewer if the root system was smaller. After 1 week incubation at 20 ºC, colonies from plated roots were counted to determine percent root infection, and the root segments were collected and dried at 100 ºC. The dry weight of the entire root system for each plant was recorded. Total inoculum produced over the test period by a plant could be divided by dry root weight to derive CFU/dry root weight.

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[1] A version of this paper was presented at the Fourth Sudden Oak Death Science Symposium, June 15-18, 2009, Santa Cruz, California.

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To compare the amount of inoculum produced on plant roots by *P. ramorum* to that of *Phytophthora* species specialized as root pathogens, a plant host other than *V. tinus* was required. Rhododendron is susceptible to a number of *Phytophthora* root rots; therefore, *Rhododendron* 'Cunningham's White' was chosen, since it could be easily rooted in Turface and used in such experiments.

Experiments

To examine the amount of inoculum given off by inoculated roots of *V. tinus* over 7 weeks, runoff from eight inoculated plants was sampled weekly over 50 days in three trials. Peak inoculum production was observed at 1 to 3 weeks after inoculation. After 50 days, *Viburnum* roots showed some browning, and percent colonization was 12 to 26 percent.

To look at the effect of root age on amount of inoculum in runoff, cuttings were rooted 2 weeks apart and inoculated when the youngest cuttings first produced sufficient adventitious roots. Runoff was collected every 3 days for 16 days. More inoculum was produced on young- (48 to 56 day) and medium-aged (62 to 70 day) cuttings compared to older (79 to 86 day) cuttings in three trials. Percent root colonization did not differ among treatments.

When infected cuttings were incubated at different temperatures for 16 days in three trials, both regression analysis and an AOV of total CFU/dry root weight showed that roots incubated at 25 ºC gave off significantly smaller amounts of inoculum. Root colonization was significantly higher on plants at 15 ºC than at 20 or 25 ºC.

When four species of *Camellia* grown from seed were compared to *Viburnum* cuttings in three trials, root-infected *Viburnum tinus* gave off significantly greater amounts of inoculum than *Camellia* species.

We also compared the amount of inoculum in runoff from *P. ramorum*-infected *Rhododendron* cuttings to that of cuttings infected with *P. cactorum* and *P. citricola*, finding little difference in three trials, although percent root infection was significantly lower for *P. ramorum* than the other two *Phytophthora* species.
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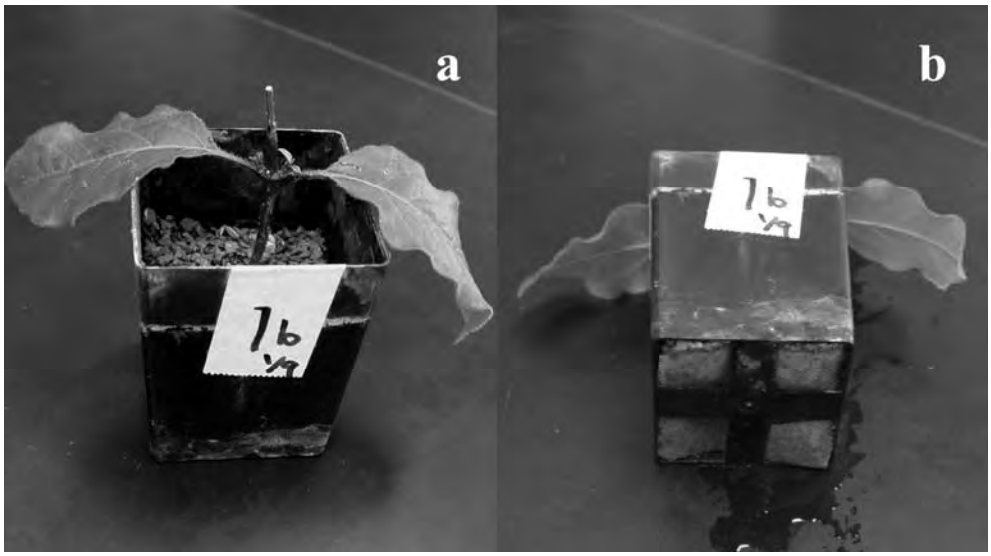

Figure 1—A test system to study the production of inoculum by plant roots infected with *P. ramorum*. Infected plants are transplanted into 2x2 inch plastic pots containing uninfested Turface® potting media and lined at the bottom with a plastic mesh allowing good drainage. a) A pot containing a rooted cutting of *Viburnum tinus*. b) A pot on its side displaying the mesh insert.

Conclusion

These results suggest the importance of infected roots in the life cycle of the pathogen, since root infection is difficult to detect, but inoculum in runoff might result in spread of the pathogen

Biology I

Persistence of *Phytophthora ramorum* and *Phytophthora kernoviae* in U.K. Natural Areas and Implications for North American Forests[1]

Elizabeth J. Fichtner,[2] David M. Rizzo,[2] Joan Webber,[3] Susan A. Kirk,[3] and, Alistair Whybrow[3]

Abstract

Phytophthora kernoviae (*Pk*) and *Phytophthora ramorum* (*Pr*) are recently introduced pathogens in United Kingdom (U.K.) woodlands. *Pk* is also an emerging threat to coastal heathland where it infects *Vaccinium myrtillus*. In infested woodlands, an invasive plant, *Rhododendron ponticum*, supports sporulation of both pathogens, providing primary inoculum for infection of *Fagus sylvatica*. *R. ponticum* has been removed from several infested woodlands; however, the long term efficacy of *R. ponticum* removal for disease management is unknown. The epidemiology of *Pk* in infested heathland is not yet understood. The aggressiveness of *Pk* in U.K. woodlands and heathland elevate the biosecurity concern associated with its potential introduction to North American forests.

The potential for *Pr* and *Pk* to infect and roots in woodlands was investigated. Roots and associated rhizosphere soil, overlying leaf litter, and foliage were collected from *R. ponticum*-invaded woodlands and woodlands cleared of the invasive plant. In *R. ponticum*-invaded woodlands, adventitious, layered roots of *R. ponticum* were excavated, whereas roots of emergent *R. ponticum* seedlings and mature *F. sylvatica* trees were sampled in cleared woodlands. Both *Pr* and *Pk* were baited from surface-sterilized *R. ponticum* roots and associated leaf litter, but not from rhizosphere soil in uncleared woodlands. In cleared woodland, *Pk* was baited from surface sterilized roots of *R. ponticum* seedlings and mature *F. sylvatica*.

In *Pk*-infested heathland, symptomatic *V. myrtillus* was collected and rhizomes, roots, shoots, and foliage were independently sampled for isolation of *Pk*. Furthermore, roots, stems, and foliage of nursery-reared *V. myrtillus* were inoculated with *Pk* for completion of Koch's Postulates. Although *Pk* was isolated from all plant parts, only leaves and stems were symptomatic. Sporangia were abundant on inoculated foliage, and were observed on the surface of inoculated, asymptomatic root and rhizome tissues. The prolific foliar sporangia production followed by leaf abscission suggest that the pathogen may spread long distances in coastal winds, yet asymptomatic root and rhizome infections may support local spread and survival.

To assess the risk of *Pk* to North American forests, leaves and roots of *Umbellularia californica*, *R. occidentalis*, and *R. macrophyllum* were inoculated with *Pk* for determination of disease incidence and severity, and sporulation potential. As a positive control, *V. myrtillus* and *R. ponticum* were concurrently inoculated. All plants were susceptible to *Pk* and

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supported sporangia production. Oogonia were produced on leaves and roots of *U. californica*. These preliminary data support the proposed threat of *Pk* to North American forests and suggest the necessity for enhanced biosecurity measures to prevent pathogen introduction and establishment.

Symptoms Associated with Inoculation of Stems on Living Douglas-fir and Grand Fir Trees with *Phytophthora ramorum*[1]

Gary Chastagner,[2] Kathy Riley,[2] Katie Coats,[2] Marianne Elliott,[2] Annie DeBauw,[2] and Norm Dart[3]

Abstract

To obtain a better understanding of the potential risk of infection and colonization of living Douglas-fir (*Pseudotsuga menziesii*) and grand fir (*Abies grandis*) stems, the stems on over 150 trees of each species were inoculated at a Christmas tree farm near Los Gatos, California. This study had the following objectives: 1) Determine if inoculation timing affects the infection of living Douglas-fir and grand fir stems, 2) Characterize symptom development associated with inoculated stems, and 3) Determine what stem tissues were colonized by *P. ramorum*.

Trees were inoculated during fall 2007, late winter 2008, and summer 2008 by using a cork borer to remove a bark plug from the stem on opposite sides of each tree. On each host, a mycelial plug of an NA1 isolate of *P. ramorum* from that host was then placed in one hole and a plug of plain agar media was placed in the other hole. The bark plugs were replaced, sealed with Vaseline, and covered with duct tape. The average stem diameter at the point of inoculation was 5.9 cm (Douglas-fir) and 6.2 cm (grand fir). Field data on symptom development have been taken at approximately 4-month intervals since inoculation. The colonization of stem tissue was evaluated by periodically dissecting a representative subset of inoculated stems. Bark and woody tissues were surface sterilized for 30 seconds in a 1:9 dilution of bleach and plated onto CARP selective medium. Surface sterilized bark and woody tissues were also subjected to qPCR testing.

Four months after inoculation of the Douglas-fir, there was no difference in the percentage of trees that had cankers regardless of whether they were inoculated in the fall or winter (fall – 90.2 percent and winter – 90.5 percent). Most of the fall- and winter-inoculated trees exhibited extensive resin flow. Branch flagging in the area of the cankers was evident on a few trees. On the fall-inoculated trees, there was a slight increase in canker length between March and July. By July, the average canker length on the winter-inoculated trees was about half the size of the cankers on the fall-inoculated trees (4.8 cm vs. 11.6 cm). The longest canker on the fall- or winter-inoculated trees was 47 cm long and cankers had girdled 12.5 percent of the fall-inoculated trees. One of these girdled trees died during spring 2009. No cankers were observed on any of the check plugs.

The size of cankers on the grand fir stems were much smaller than those that developed on the Douglas-fir. Four months after inoculation, about twice as many of the trees inoculated in the winter (76.8 percent) had visible cankers compared to those inoculated in the fall (34.0 percent). Although some cracking of the bark was observed in the canker area, very little resin

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flow was observed. On the fall-inoculated trees, the average canker length was still only about 2.0 cm 1 year after inoculation. On the winter-inoculated trees, the average length of cankers in July 2009 was 4.9 cm; 1 year after inoculation (March 2009), canker lengths had increased slightly to 6.4 cm. The largest canker on any of the inoculated trees was 25.7 cm long. None of the cankers have girdled any of the grand fir trees and no cankers were observed on any of the check plugs.

No cankers developed on any of the trees that were inoculated during the summer. In addition, limited expansion of the cankers on trees that were inoculated in the fall or winter has occurred since July 2008.

Isolation from bark and wood has resulted in very limited recovery of *P. ramorum*. The pathogen was recovered from 1.4 percent of 1,780 isolations from Douglas-fir and 0.2 percent of 1,418 isolations from grand fir. qPCR positives from surface sterilized tissues were restricted to symptomatic bark and the woody tissue just beneath the bark or a thin layer of discolored tissue between the 2007 and 2008 wood below cankers. Histological studies are in progress to determine what tissues are colonized and what pathogen structures are present in those tissues.

Acknowledgments

This research was financially supported by the U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station, and the Pacific Northwest Christmas Tree Association. The assistance of Robbie Criswell and Ann Northrup is gratefully acknowledged.

Effects of *Phytophthora ramorum* Infection on Hydraulic Conductivity and Tylosis Formation in Tanoak Sapwood[1]

Jennifer Parke,[2] Bradley Collins,[3] Barb Lachenbruch,[4] and Everett Hansen[3]

Abstract

Tanoak (*Lithocarpus densiflorus*) is highly susceptible to sudden oak death caused by *Phytophthora ramorum.* Symptoms include dying crowns, bleeding cankers, and, eventually, death of infected trees. The cause of mortality is not well understood, but we showed previously that naturally infected mature trees have reduced sap flow and reduced hydraulic conductivity. One possible mechanism for this is the presence of tyloses in xylem vessels formed in response to *P. ramorum* infection.

In this study, we examined the development of tyloses in relation to hydraulic conductivity of *P. ramorum*-infected sapwood. Boles of understory tanoak trees (6 to 12 cm DBH) were inoculated with mycelial plugs of *P. ramorum* placed at the cambium. Wounded non-inoculated controls received agar plugs without the pathogen. Trees were felled at one of two sampling dates (4 months or 14 months after inoculation) and boles were cut into subsections. Three subsections (0 to 12 cm and 12 to 24 cm above the inoculation point, and 12 to 24 cm below the inoculation point) were used for assessment of specific conductivity and observation of tyloses. Specific conductivity was measured in the lab on 1 cm x 1 cm x 10 cm pieces of wood chiseled from the sections. Sapwood was viewed in cross-section at 100 x, and the proportion of vessel lumens with tyloses was determined.

At both sampling dates, inoculated trees with xylem infections had significantly more tyloses as compared to wounded controls. At 14 months after inoculation, for all three bole sections combined, the proportion of vessel cross-sections with tyloses was 29.1 percent for diseased trees and 0.4 percent for wounded control trees ($p < 0.001$). Tylosis frequency was strongly associated with a decrease in specific conductivity of sapwood tissue ($p < 0.0001$, $r^2 = 0.24$). Over time, tylosis development increased in tissues further away from the inoculation site in advance of the vertical spread of infection. Results suggest that *P. ramorum*-infected sapwood contains numerous tyloses that could significantly impede stem water transport.

Results from this work are reported in more detail in: **Collins, B.R.; Parke, J.L.; Lachenbruch, B. and Hansen, E.M. 2009.** The effects of *Phytophthora ramorum* infection on hydraulic conductivity and tylosis formation in tanoak sapwood. Canadian Journal of Forest Research. 39(9) 1766–1776.

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Virulence, Sporulation, and Elicitin Production in Three Clonal Lineages of *Phytophthora ramorum*[1]

Daniel Manter,[2] Everett Hansen,[3] and Jennifer Parke[3]

Abstract

Phytophthora ramorum populations are clonal and consist of three clonal lineages: EU1 is the only lineage found in Europe with a few isolated nursery infections in the USA; NA1 is associated with natural infestations in California and Oregon as well as some nursery infections in North America, and NA2 has a limited distribution and has only been isolated from a few nurseries in the USA. Recent studies have shown that the clonal lineages may have varying degrees of aggressiveness on some host species, such as *Quercus rubra*. In this study, we examined virulence, sporulation, and elicitin production of five *P. ramorum* isolates from each of the three clonal lineages. Virulence (lesion size) and sporulation (sporangia production) were determined on inoculated detached leaves of rhododendron 'Nova Zembla'. Elicitin production was determined *in vitro*.

Lesion area differed between the clonal lineages ($p < 0.001$). Leaves inoculated with EU1 and NA2 isolates had significantly greater lesion area than NA1-inoculated leaves (approximately 4.2, 3.6, and 0.8 cm^2 respectively). Similarly, sporangia production was greatest in the EU1 and NA2 isolates compared to the NA1 lineage; however, considerable variation was noted between trials. In trial one, sporangia production was very low and there was no difference between clonal lineages ($p = 0.20$); however, there were significant differences among isolates ($p = 0.04$). Sporangia production was much greater in trial two than in trial one, likely because the leaves were misted more frequently in the 1 to 2 days before processing to increase sporulation. In trial two, there were significant differences among clonal lineages and isolates ($p = 0.0003$). Lineages EU1 and NA2 produced significantly more sporangia per leaf ($p < 0.001$) than did lineage NA1 (approximately 800, 1000, and 300 sporangia per leaf respectively).

Real-time PCR assays detected expression of both Class I elicitins (Ram-α1 and Ram-α2) in all 15 isolates. Of the two elicitins, only the Ram-α2 differed between lineage ($p = 0.000$) with nearly 2-fold higher levels of expression in the EU1 and NA2 lineages as compared to the NA1 lineage. Ram-α2 expression showed a positive linear relationship with isolate virulence or lesion size ($r^2 = 0.71$). The significant, positive, linear relationship was also observed between Ram-α2 expression and sporulation although it was not as strong ($r^2 = 0.21$).

In conclusion, isolates belonging to clonal lineages EU1 and NA2 are generally more virulent, produce more sporangia, and produce more Ram-α2 elicitin *in vitro* than do isolates belonging to lineage NA1. This suggests that Ram-α2 may contribute to the fitness of *P. ramorum*. Further studies are needed to determine what quantities of Ram-α2 are produced *in planta* and any additional factors that may directly influence its activity.

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California Bay Laurel Susceptibility to *Phythophthora ramorum* Depends Upon Season, Leaf Age, and Fungal Load[1]

Steve Johnston,[2] Nathan Rank,[2] Michael Cohen,[2] and Ross Meentemeyer[3]

Abstract

Phytophthora ramorum can produce spores on dozens of native California plant species, but the most important vector for infection of oak (*Quercus*) is California bay laurel (*Umbellularia californica*). Presence of bay laurel is associated with increased infection of oaks and it is the most common tree species that co-occurs with coast live oak *(Quercus agrifolia)* in northern California woodlands. Understanding the interaction between *P. ramorum* and bay laurel is critical to predicting dynamics of disease establishment in mixed oak woodlands. Previous studies showed that *P. ramorum* lesion growth and expression of *P. ramorum* symptoms depends on environmental factors and on bay laurel genotype. However, these studies did not measure susceptibility to infection by *P. ramorum* spores on the leaf surface. In addition, they did not work with pathogen isolates that had been cleared of all microbial associates.

We addressed five questions using detached leaf assays employing *P. ramorum* isolates and bay laurel trees: 1) How does leaf age affect susceptibility to infection? 2) Do *P. ramorum* isolates vary in infectivity of bay laurel (pathogen isolate by host tree interaction)? 3) What traits of individual bay laurel relate to susceptibility? 4) How does susceptibility vary over the year? and 5) At what temperatures are trees most susceptible to infection? For this study, we used 20 *P. ramorum* isolates from across the contiguous range in California and 51 bay laurel trees from eastern Sonoma County, California, where infection of bay laurel and canker host species is widespread.

Young leaves of bay laurel from the current growing season are less susceptible to infection than leaves that grew in prior years (Figure 1). This effect was observed for six different host trees. Lesion scores were twice as high on leaves from previous growing seasons than on current year leaves. We also found that individual bay laurel trees vary significantly in susceptibility, and *P. ramorum* isolates vary in infectivity of bay laurel. However, there was also a significant host tree by pathogen isolate interaction. Trees that were not susceptible to most *P. ramorum* isolates were very susceptible to a few, and some *P. ramorum* isolates that were usually unable to infect a bay laurel tree frequently infected a few host trees.

To identify which bay laurel traits affect susceptibility, we quantified susceptibility on 24 trees from three Sonoma county localities exposed to four *P. ramorum* isolates. We assessed four leaf characteristics, including leaf size and protein content; eight characteristics of trees growing in the field, including leaf retention, number of lateral shoots, and insect herbivory; features of the field site where the trees naturally grow, including topographic position, temperature-moisture index, and microclimate conditions recorded by a data logger; and the

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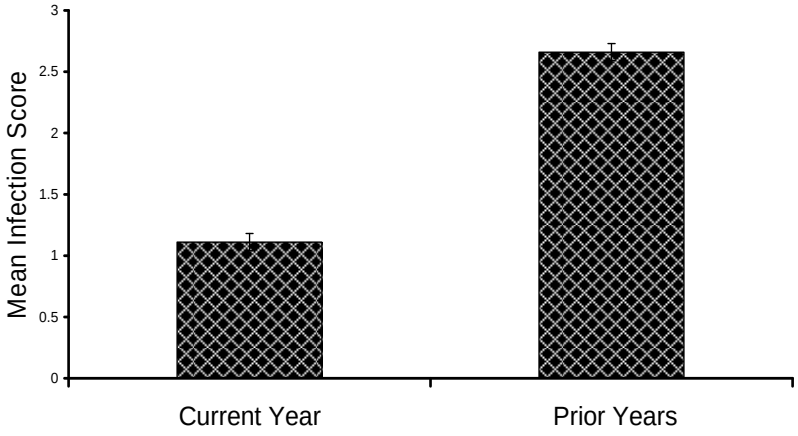

Leaf Age p , 0.037, Leaf Age*Isolate p < 0.0001

Figure 1. Current year bay laurel leaves are less susceptible to *P. ramorum* Infection than older leaves.

natural microbial community growing on bay leaves, including the diversity and total number of fungal and bacterial colony forming units (CFU). To relate these characteristics to susceptibility, we conducted a path analysis based on *a priori* hypotheses about direct and indirect relationships among predictor variables. Results indicated that leaf area and the number of lateral shoots were positively related to susceptibility, while fungal CFU was negatively related to it. Temperature moisture index and maximum relative humidity were positively related to fungal load.

Previous studies have shown that *P. ramorum* sporulates at relatively low temperatures, which are common during winter rain in California, but that bay laurel infection occurs later in spring when conditions are warmer. We examined susceptibility at the same temperature range and found that susceptibility was highest between 5º C to 15 ºC. These results support the finding that the pathogen is capable of growth and sporulation at the lower temperatures that prevail during winter, but they do not explain why bay laurel infection levels remain low until late spring.

Other studies suggest that bay laurel is less susceptible during the summer months, and our susceptibility assay findings agreed with this conclusion. We found that susceptibility declines during summer and early fall and increases slightly during fall and winter. Shortly before bud break, susceptibility increases rapidly and it peaks in May or June.

We propose a new conceptual model for *P. ramorum* epidemiology in oak woodlands (Figure 2). Once winter rains begin, *P. ramorum* begins to produce spores, but the bay laurel trees are not yet susceptible enough to support multiple cycles of spore production. In years when late spring rains overlap with increased susceptibility of bay laurel trees, cascading spore production facilitates bay laurel infection. Our results highlight the importance of bay laurel phenology in disease establishment.

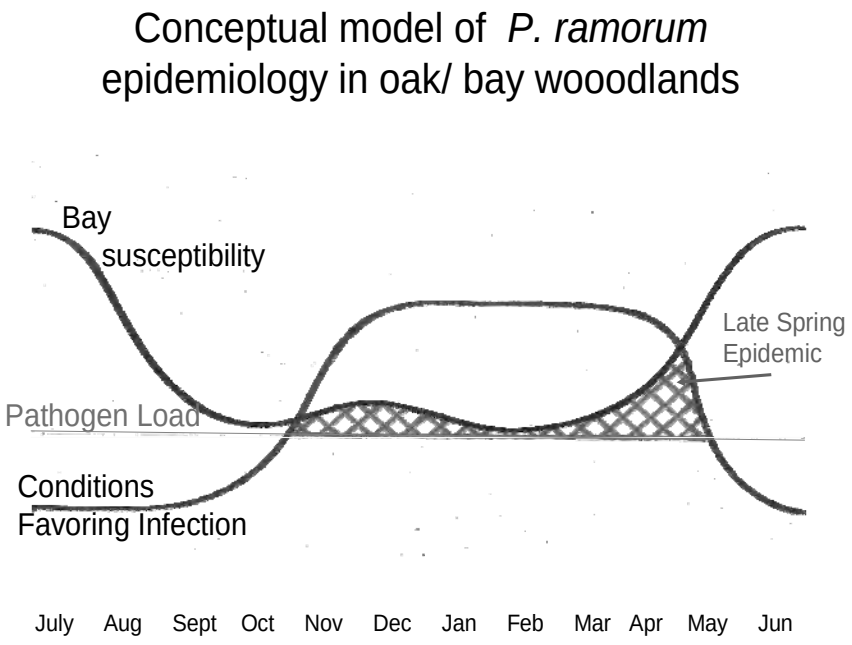

Figure 2. Model for *P. ramorum* epidemiology in oak woodlands.

Diagnostics and Mycology

ELISA and ImmunoStrip® for Detection of *Phytophthora ramorum*, *P. kernoviae*, and Other *Phytophthora* Species[1]

Francisco. J. Avila,[2] Barbara Schoedel,[2] Z. Gloria Abad,[3] Michael D. Coffey[4] and, Cheryl Blomquist[5]

Abstract

The goal of this work was to develop improved tools for the detection of *Phytophthora ramorum* and *P. kernoviae* for field and the laboratory use. ImmunoStrip® and ELISA were selected as the test formats for development. Presently, the diagnosis of sudden oak death (SOD) in the national survey of *P. ramorum* depends on the use of ELISA to pre-screen samples, and then confirms results with PCR and morphological identification. This approach has some disadvantages because the ELISA has a wide spectrum reaction with *Phytophthora* spp. and cross-reacts with *Pythium* spp. A faster and more specific serological test to detect *P. ramorum* would be very useful in the survey of this pathogen. *P. kernoviae* was first detected in rhododendron in Cornwall in 2003 during surveys for *P. ramorum*, and the pathogen has been found in New Zealand. *P. kernoviae* has not been reported in the U.S. However,

Mycelium suspensions of *P. ramorum* isolates from California, Washington, and Europe, as well as mycelium suspension of *P. kernoviae* from Europe, were injected into mice for monoclonal antibody production and in rabbits for polyclonal antibody production. The resulting antibodies were screened using a collection of *Pythium* and *Phytophthora* that included *P. kernoviae*, and *P. ramorum* (from U.S. and Europe); and healthy plants such as red pine (*Pinus resinosa*) needles, rhododendron (*Rhododendron* sp.), and oak (*Quercus*) leaves. Antibodies with highest sensitivity and specificity to *P. ramorum* and *P. kernoviae* and without healthy tissue reaction were considered candidates for immunoassay development. Although many new antibodies were produced, none of them were species-specific to *P. ramorum* or *P. kernoviae* and cross-reacted with other species of *Phytophthora*.

Several ImmunoStrip® and ELISA prototypes were developed. Each was evaluated with pure cultures of *Phytophthora* and *Pythium*, and *Phytophthora*-infected plant material. The resulting ImmunoStrips® were found to be sensitive, rapid, and easy to perform with field materials, and able to detect all *P. ramorum* and *P. kernoviae* isolates while not detecting any *Pythium* isolates. One strip prototype was not very reactive with mycelium younger than 5 weeks old; however, another prototype with a different antibodies pair detected *P. ramorum* and *P. kernoviae* in all samples tested.

Some newly developed antibodies when used in ELISA recognized *P. ramorum* and *P. kernoviae*, detected a smaller spectrum of *Phytophthora* species and did not cross-react with

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the *Pythium* species. Another set of antibodies in an ELISA format detected all *Phytophthora* species tested and recognized fewer *Pythium* species.

These results demonstrate that the tools for detection of *P. ramorum* can be significantly improved because some prototype tests recognize all *P. ramorum*, do not cross react with *Pythium*, and detect a significantly smaller spectrum of *Phytophthora* species. We are continuing this study including a comparison of the new tests with PCR especially with field samples.

Mating of *Phytophthora ramorum*: Functionality and Consequences[1]

Xavier Boutet,[2] Annelies Vercauteren,[3] Kurt Heungens,[3] and Anne Chandelier[2]

Introduction

Phytophthora ramorum (Werres, De Cock, Man in't Veld), which causes "sudden oak death" in the United States and dieback and leaf necrosis in ornamental plants (mainly *Rhododendron* and *Viburnum*) in Europe, is a heterothallic species with two mating types, A1 and A2 (Werres and others 2001, Rizzo and others 2002). Molecular studies on the population of *P. ramorum* isolates revealed a structure composed of three clonal lineages: one lineage from Europe (EU1) and two lineages from North America (NA1 and NA2) (Elliott and others 2009). Initial pairing studies revealed geographical separation of mating type isolates: all EU1 isolates were of A1 type whereas all NA1 and NA2 isolates were of A2 type (Werres and others 2001, Brasier 2003). However, since 2003 a few rare reports of A2 mating type isolates in Europe and reports of A1 mating type isolates in the United States (U.S.) have been made. In Europe, three EU1 A2 isolates were reported in Belgium (Werres and De Merlier 2003; Heungens, personal communication). In North America, some EU1 A1 isolates were reported in U.S. nurseries (Hansen and others 2003, Grünwald and others 2008). These findings suggest the potential for crossing between both mating types. However, attempts to produce oospores *in vitro* with classical methods were difficult compared to other heterothallic species (Werres and Zielke 2003, Brasier and Kirk 2004), therefore suggesting a weak functionality of the sexual system in *P. ramorum*. In a previous study, some critical parameters such as the gelling agent quality, the nutrient source, or the spatial arrangement of the two mating types in the Petri dish were optimized to produce a large amount of oospores *in vitro*. A particular EU1 A1 strain was found to be a better mating partner than other EU1 A1 strains when paired with some European (EU1) or American (NA1) A2 strains (Boutet and others 2009). The aims of this study were to investigate the functionality of *P. ramorum* sexual reproduction and to evaluate the characteristics of single-oospore isolates.

Materials and Methods

The *P. ramorum* strains used in this study are listed in table 1. Oospores resulting from the mating of EU1 x EU1 (2299 x 3237) and EU1 x NA1 (2299 x 3528 or 3638) strains were extracted from the agar plate after different maturation times (60, 110,

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250, 350, and 500 days) by using a newly developed method to separate oospores from mycelium and asexual spores (sporangia and chlamydospores) (Boutet and others 2010). Oospores were stained with tetrazolium bromide (MTT) as described by Sutherland and Cohen (1983) to evaluate their viability. Their germination ability was evaluated in parallel by embedding them in Soft Water-Agarose (water agarose 0.6 percent). Oospore progenies were characterized in terms of (1) genetic rearrangement using microsatellite markers (Vercauteren and others 2010) and (2) pathogenicity on *Rhododendron* "Cunningham's White" by inoculating leaves with a mycelium plug of each oospore offspring.

Table 1—List of *P. ramorum* isolates used in this study

Species	Isolate	Collection	Host	Mating Type	Lineage
P. ramorum	2299	CBS (101330)[1]	*Viburnum* sp.	A1	EU1
P. ramorum	3237	CRA-W[2]	*Viburnum bodnantense*	A2	EU1
P. ramorum	3638	ILVO (PRI 483)[3]	*Rhododendron* sp.	A2	NA1
P. ramorum	3528	USA (014)[4]	*Quercus* sp.	A2	NA1

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Results

The viability test (staining with MTT) indicated that between 20 and 70 percent of the oospores possessed viable cellular activity. In contrast, only approximately 0.2 percent of these oospores were able to germinate (fig.1), suggesting that the MTT method can not be used to estimate the germination ability.

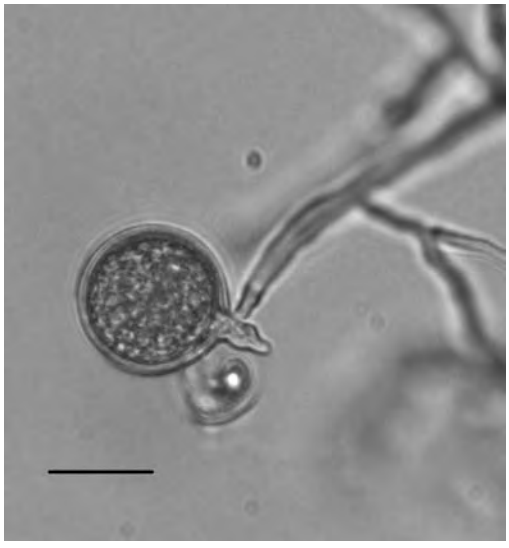

Figure 1—Germinated oospore from an EU1 x EU1 mating of *P. ramorum*. Scale bar represents 20 µm.

Among the isolates that were recovered from oospores maintained from 110 to 500 days in culture, 37 originated from the EU1 x EU1 pairing and 13 originated from EU1 x NA1 pairings. Microsatellite marker analysis showed that the progeny presented genetic rearrangements, either by allele combinations of both parents (EU1 x NA1 progenies) or by loss of one of the parental alleles due to a shift from heterozygosity to homozygosity (EU1 x EU1 progeny). Pathogenicity analysis revealed a large variability among the EU1 x EU1 single oospore isolates with more than 50 percent being significantly less aggressive on *Rhododendron* leaves than their EU1 parents (fig. 2) whereas no significant differences were found between EU1 x NA1 offspring and their parents.

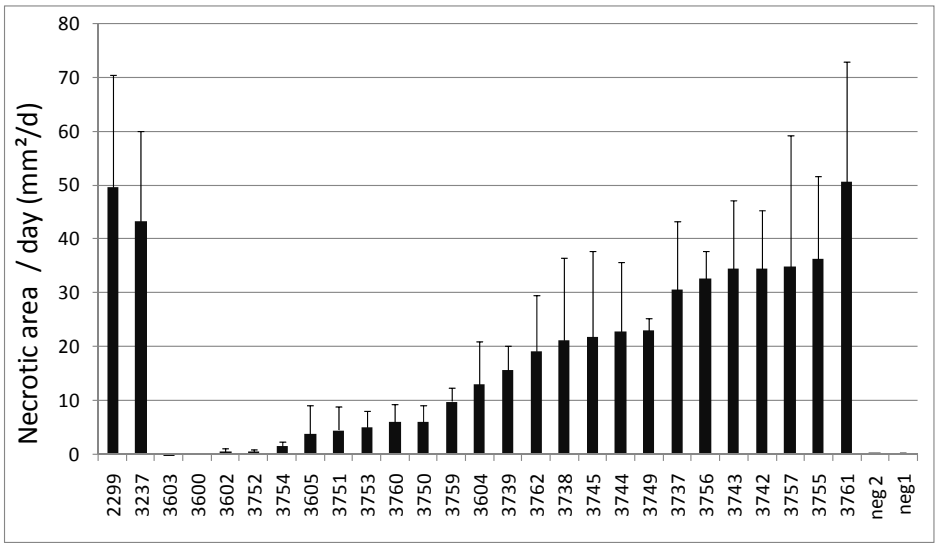

Figure 2—Pathogenicity of EU1 x EU1 offspring on *Rhododendron* leaves expressed by daily growth rate of necrotic area (three replicates). The parental strains are strains 2299 (EU1, A1) and 3237 (EU1, A2).

Conclusion

In a context of pest risk analysis, these data demonstrate the functionality of the *P. ramorum* mating system and the possibility of genetic exchange between the EU1 and NA1 populations, although the proportion of viable single-oospore isolates obtained was very low. The preliminary characterization of the progeny highlights an important phenotypical variability after only one generation. Nevertheless, there is still no data on the ability of *P. ramorum* to sustain a sexual cycle on host plants and in the natural environment. Moreover, a large proportion of EU1 or NA1 *P. ramorum* isolates seem unable to produce large amounts of oospores. Therefore, further experiments are required to fully assess the risk presented by sexual reproduction of *P. ramorum* in nature.

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Unstable Aneuploid Progenies of *Phytophthora ramorum*[1]

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Abstract

Phytophthora ramorum has three populations belonging to the same species: the two North American populations (NA1 and NA2) and the European population (EU1). All three populations are genetically distinct lineages, as revealed by different molecular marker systems. *P. ramorum* is also heterothallic, with two opposite mating types, A1 and A2. All NA1 and NA2 isolates are of the A2 mating type. All EU1 isolates have been identified as A1, except for three A2 isolates collected in Belgium in 2002 and 2003. Since 2004, no isolates of the A2 mating type have been reported in Europe, which limits the risk of sexual recombination in Europe. In contrast, several EU1 isolates have been reported in nurseries in North America, some even at sites where NA1 isolates were also found. Although sexual reproduction in nature has not been observed yet, the presence of isolates of both mating types at a single site might lead to genetic recombination, which could lead to an increase in pathogen fitness and host range. The formation of sexual resting oospores might also increase the long-term survival of *P. ramorum*. The ability of plant pathogen populations to respond to novel challenges from their environment, such as host resistance and pesticide exposure, depends on their mechanisms for generating genetic variation.

Germinating oospores have been obtained *in vitro* from crosses between EU1-A1 and NA1-A2 isolates as well as between EU1-A1 and EU1-A2 isolates (Boutet and others, unpublished), but at a low frequency. To determine the nature of sexual recombination in *P. ramorum*, germinating oospores of a cross between an NA1-A2 isolate and an EU1-A1 isolate were genotyped with microsatellite markers that were heterozygous in the parental isolates. All crosses contained alleles of both parents, confirming exchange of genetic material, but meiotic irregularities occurred frequently. Non-Mendelian inheritance events were also observed. This included inheritance of more than two alleles at a single locus or loss of alleles from one of the parents at a separate locus. The aneuploid progeny was also mitotically unstable: zoospore and hyphal tip derivatives of the progenies showed genotypic rearrangements as well as phenotypic variation. These data indicate that progenies from crossings between *P. ramorum* isolates are often aneuploid and genetically unstable.

Introduction

Phytophthora species are known to exhibit phenotypic variation during asexual growth. This is due to genome instability caused by transposable elements, gene conversion, mitotic recombination, and/or dispensable chromosomes (Chamnanpunt and others 2001, Francis and St. Clair 1997, Goodwin 1997, Judelson 2002). Additional variation can be created if the sexual cycle in *P. ramorum* is functional;

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exchange of genetic material from both parents can result in an increased fitness and host range. Non-Mendelian inheritance is not uncommon in *Phytophthora* species (Förster and Coffey 1990, Carter and others 1999, Dobrowolski and others 2003, Judelson 1996, Judelson and others 2002, Van der Lee and others 2004). Sexual progeny of *P. cinnamomi* and *P. sojae* were also mitotically unstable. Sectoring in colonies has been seen in progeny of *P. cinnamomi* (Zheng and Ko 1996, Dobrowolski and others 2003). This morphological variation could be associated with the loss of alleles in the asexually derived subcultures. Chamnanpunt and others (2001) reported that in hybrid strains of *P. sojae*, unique genetic types could be rapidly generated by mitotic gene conversion. Mitotic gene conversion resulted in the reassortment of loci that show close linkage during meiotic recombination at remarkably high frequencies, as high as 3.10^2 conversions per locus per nucleus per generation. The objective of this study was to determine whether this type of genetic instability is also observed in progeny of *P. ramorum*.

Materials and Methods

In total, 41 oospore progenies from pairings between complementary *P. ramorum* isolates were analyzed with microsatellite markers, among which 28 progenies originated from EU1 x EU1 pairings, and 13 originated from EU1 x NA1 pairings. Separate subcultures were made from five different growth sectors within the colony of an EU1 x NA1 progeny. Separate single zoospore cultures were also created from different progeny. DNA was extracted from each subculture. The microsatellite marker profiles of the parents, the original progeny, and all subcultures were determined as described in Vercauteren and others (unpublished). Eight loci (KIPrMS18, KIPrMS64, KIPrMS82a-b, ILVOPrMS133, and ILVOPrMS145a-c) were explored for allelic rearrangements. When no difference between the progeny and the parents was detected for these loci, extra microsatellite markers were used for further genotyping.

Results and Conclusions

Microsatellite analysis revealed that each of the 13 EU1 x NA1 progenies contained alleles of both parents. In 20 EU1 x EU1 progenies, loss of heterozygosity was observed for at least one of the eight microsatellite loci analyzed. These progenies show the loss of at least one allele from the loci that are heterozygous in the parents as a result of becoming homozygous. For the eight other EU1 x EU1 progenies, up to seven extra microsatellite markers were used, after which rearrangements were observed in all of them. Therefore, microsatellite fingerprinting profiles showed genetic rearrangements in all progenies. Based on this data there is evidence that all progenies were recovered from germinating oospores and that the sexual cycle in *P. ramorum* is functional, with an exchange of genetic material from both parents. Selfing was not detected in any of the EU1 x NA1 progenies. Non-Mendelian inheritance of alleles was common. In all EU1 x NA1 progenies at least one locus contained alleles from only one parent or had more than two alleles.

Mycelial colonies from germinating oospores often contained sectors with a reduced growth rate. Microsatellite marker analysis confirmed genetic rearrangements in these sectors. The separate single zoospore cultures derived from one progeny also had differences in allele number at some loci. However, genotypic changes may have

occurred prior to zoosporogenesis, during growth of the subculture. Genotypic differences in these single-zoospore subcultures were correlated with phenotypic differences. In contrast to the genetic rearrangements observed in the mycelium and zoospore cultures derived from the progeny, no such rearrangements were observed in the parental strains, even after years of storage and subculturing.

The likelihood of sexual recombination of *P. ramorum* in nature is very low, but the risk remains. Mitotic gene rearrangements after sexual recombination may result in a wide genetic diversity. This could then lead to the development of strains that are adapted to new environmental challenges or different hosts.

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Genetics and Resistance

Tracking Populations and New Infections of *Phytophthora ramorum* in Southern Oregon Forests[1]

Jennifer Britt,[2] Simone Prospero,[3] Niklaus Grünwald,[4] Alan Kanaskie,[5] and Everett Hansen[2]

Abstract

Since the discovery of *Phytophthora ramorum* in southern Oregon forests in 2001, newly infested areas are located each year. We tracked the spread and dispersal using DNA fingerprinting. While among site genetic variance was low, we did find changes in genotype presence and frequency at the site level. These genotypic differences allowed us to map the spread of some individual genotypes demonstrating long and short distance dispersal of P. *ramorum* in Oregon Forests.

Previous genetic work has shown the Oregon forest population belongs to the North American one clonal lineage, it is distinct from the Oregon nursery populations, and has low genetic diversity within Oregon forests (Ivors and others 2006, Prospero and others 2007). Although the molecular diversity is low, we can use DNA markers to track the spread of P. *ramorum* in Oregon forests and give insight into how the pathogen is spreading.

Using microsatellite markers, our study aims to answer the following:
Do the new infections represent novel introductions or come from previously infested areas?
Does the genetic infection pattern suggest mode(s) dispersal?

We collected samples from a variety of hosts, including: tanoak (*Lithocarpus densiflorus*), rhododendron (*Rhododendron* sp.), evergreen huckleberry (*Vaccinium ovatum*), Oregon myrtle (*Umbellularia californica*), and poison oak (*Toxicodendron diversilobum*), and from streams and soil from 2001 through 2008. We plated the samples onto *Phytophthora* selective CARP medium in the field and/or laboratory and grown cultures out in the laboratory for identification (Hansen and others 2005). The southern Oregon range of P. *ramorum* was delineated into sites based on stream watershed, topography, and in some cases, distance. We genotyped a total of 1925 individual isolates at 5 microsatellite loci PrMS39, PrMS43, and PrMS45 (Prospero and others 2004), and PrM82 (Ivors and others 2006) on an ABI 3100 DNA sequencer. All isolates of any genotype but the most common, and approximately 25 percent of the most common isolates, were re-extracted and/or re-genotyped. Data was analyzed using the software package GenAlEx6 (Peakall and Smouse 2006).

We identified 68 novel multilocus genotypes (MGs) with 10 to 35 MGs found in each year. While the majority of MGs were present in very low numbers (< 1 percent), one MG was

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dominant in all years representing 35 to 65 percent of isolates. Six genotypes were present in all 8 years while 31 genotypes were present in only 1 year. We found no MGs matching any found in Oregon nurseries or in California.

A population assignment test, that uses expected genotype frequencies across loci to assign individuals to their population or a different population, revealed very little population structure among designated populations. Fifteen percent of individuals were assigned to their "self" population while 85 percent were assigned to an "other" population.

Despite low overall genetic variability in *P. ramorum* among sites in southern Oregon, differences in MGs among individuals are informative in tracking the spread through the forest. Figure 1 shows the different genotypic frequencies at the 35 delineated sites.

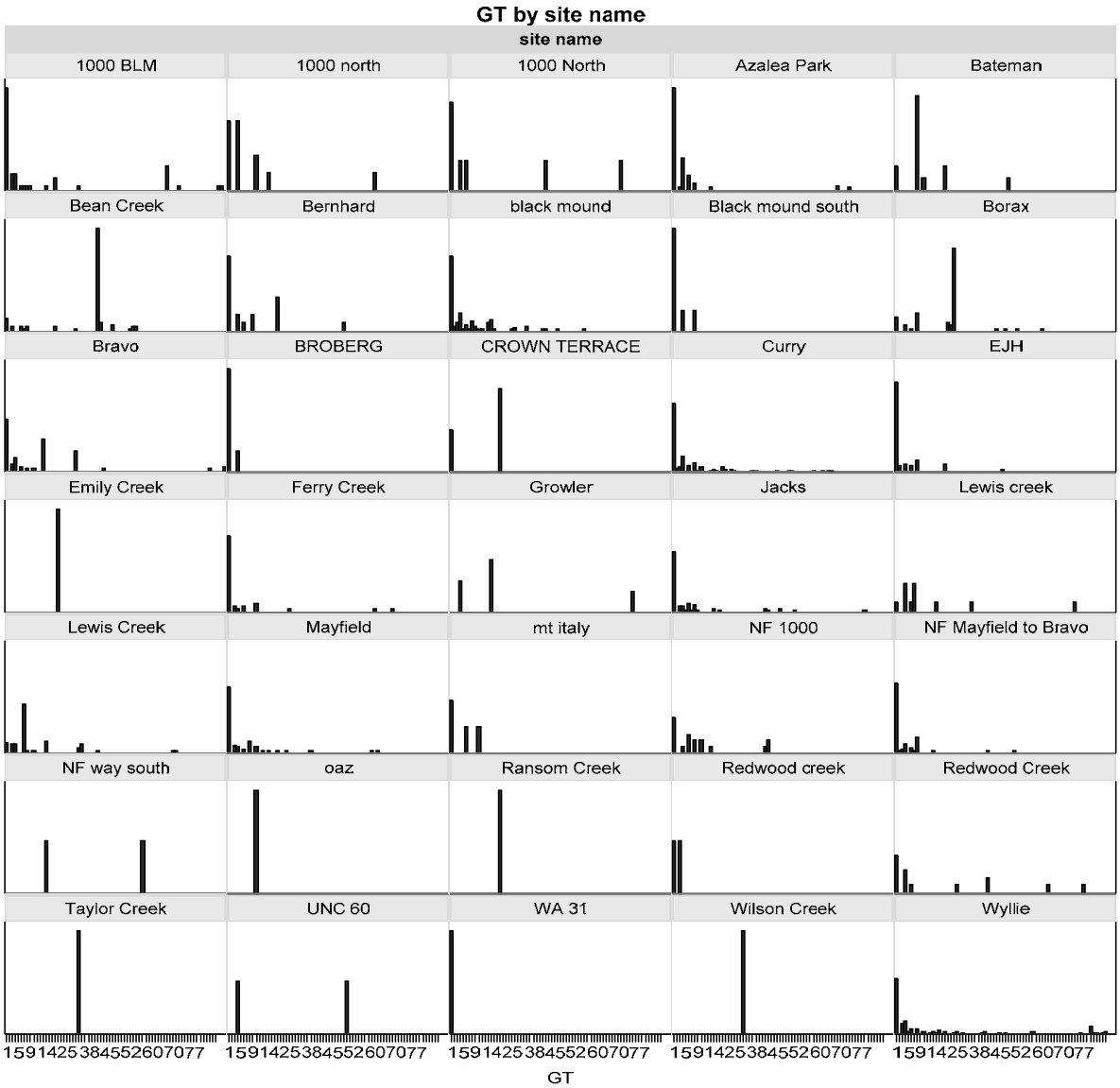

Figure 1—Multilocus genotype frequencies at 35 sites in southern Oregon forests.

What is most interesting is the dominance of different genotypes among sites. For example, MG 41 is present in over 50 percent of samples from Bean Creek, but is absent in all but a few samples at two other nearby sites. We can also see that the most common genotypes are

present at most of the sites. When this same information is mapped among sites, dispersal distances can be determined. Site level population multilocus genotypic differences supports the theory of wind and splash dispersal events where individual genotype(s) are dispersed by wind to start a new subpopulation and spread in small areas by splash dispersal.

In summary, DNA fingerprinting allows us to track new and existing *P. ramorum* infections in Oregon forests, with no new infections from California, Washington, or nurseries detected since 2008. We are also able to map how the pathogen is spreading through the Oregon forest, suggesting long distance dispersal by wind and short distance dispersal by splash events.

Our current efforts are aimed at examining the fitness of the most common genotypes in an effort to parse apart whether the dominance of one genotype is based on a founder event or the advanced fitness of that particular genotype.

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Genetic Diversity of *Phytophthora ramorum* in Belgium[1]

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Abstract

Phytophthora ramorum is thought to be an introduced pathogen in North America and in Europe based on the presence of only three clonal lineages. The North American lineages (NA1 and NA2) are responsible for infections in North American forests and nurseries, while the European lineage (EU1) is responsible for infections in Europe, mostly in nurseries. There have also been a few isolated findings of the EU1 lineage in North American nurseries. *P. ramorum* is heterothallic, with two opposite mating types, A1 and A2. The A1 mating type was originally only found in Europe, with the exception of three A2 isolates of the EU1 lineage collected in Belgium in 2002 and 2003. Although there is no evidence for sexual reproduction in nature, the presence of both mating types at a single site might lead to genetic recombination.

To verify the hypothesis of asexual reproduction in nature, and to verify the hypothesis that *P. ramorum* was recently introduced in Belgium and therefore possesses only a limited amount of genetic diversity, the Belgian *P. ramorum* population was screened with AFLP and SSLP markers. Use of the AFLP method with five primer combinations on a selected number of isolates (80) revealed 13 polymorphic fragments. These markers identified eight isolates that differed from the main genotype by one to three polymorphisms. Use of SSLP with existing microsatellite markers revealed a limited number of polymorphisms in the EU1 population. Additional microsatellite markers were then sought. A total of 146 candidate polymorphic microsatellites were prescreened using 10 isolates belonging to different EU1 genotypes. This resulted in two new primer pairs that amplify a total of three polymorphic loci, of which one was very useful. Seven markers (four existing and three new) were used to screen all 411 Belgian isolates. In total, 30 genotypes were identified, but 68 percent of the isolates belong to the main genotype EU1MG1. Although indications of accumulated mutation events were present, the overall level of genetic diversity within these isolates of *P. ramorum* appears to be limited, indicating a relatively recent clonal dispersion of the pathogen. Most of the genotypes were site-specific and some of them were detected over a period of several years at a single site, sometimes discontinuously. This indicated (latent) survival of the pathogen at those sites and led to questions about the efficiency of the eradication measures. No marker recombination was observed, indicating that no sexual recombination was found in nature.

Introduction

Since 2002, European Union (EU) emergency phytosanitary measures have been taken to prevent the introduction and spread of *Phytophthora ramorum* in Europe. In Belgium, these measures are implemented by the National Plant Protection Service (FAVV), which conducts annual inspections at all nursery and retail sites that house

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potential host plants. They also conduct surveys at parks and forests for symptoms of *P. ramorum*. Since 2002, *P. ramorum* has been detected in approximately 60 nurseries, and on new *Rhododendron* plantings in two public parks and five private gardens. Quarantine measures were implemented at all sites and the isolates have been stored.

All isolates belong to the A1 mating type, with the exception of three A2 isolates of the EU1 lineage collected in 2002 and 2003. Although there is no evidence of sexual reproduction in nature, the presence of both mating types at a single site might lead to genetic recombination.

Molecular marker systems, such as AFLP and microsatellite polymorphisms (SSLP), have been used to determine the genetic diversity within *P. ramorum* populations and to verify the presence of sexual recombination events (Ivors and others 2004, 2006, Mascheretti and others 2008, Prospero and others 2004, 2007).

However, the microsatellite markers that are currently available show few polymorphisms in the EU1 clonal lineage. The first objective of our research was thus to identify novel, polymorphic microsatellite loci in the EU1 lineage. We then used the newly developed and existing microsatellite markers, as well as AFLP, to determine the genetic diversity of the Belgian EU1 population. Indications of genetic recombination in this population were verified with the microsatellite markers. Another objective of the research was to relate the data on genetic diversity with those of isolate geographical origin, isolation year, and fungicide resistance, and to use this information to evaluate the success of the eradication efforts.

Material and Methods

All Belgian isolates collected during 2002 to 2008 were used in this study. A selection of 80 isolates was used for the comparison between AFLP and microsatellite analysis, using a stratified sample based on isolation year, host, and site. AFLP reactions were performed as described by Ivors and others (2004). For the microsatellite analysis, extra markers were needed to have a high-resolution screening. The first set of candidate microsatellite markers were 34 primer pairs designed by Ivors and others (2006) and Prospero and others (2004, 2007), which showed polymorphism within the NA1 population, but had not been screened yet for polymorphism within EU1 isolates. The second set of candidate microsatellite markers consisted of 71 dinucleotide, 27 trinucleotide, 11 tetranucleotide, one pentanucleotide, and six hexanucleotide repeat loci that were selected by screening the genome of *P. ramorum* (Tyler and others 2006) for repeats. To avoid the costs of genotyping with individual fluorescently labeled primers, universal fluorescent labeling was used (Shimizu and others 2002). For each microsatellite primer pair, polymorphism was assessed between an EU1 (PR/D/04/284) and an NA1 isolate (PRI483). This was the first selection criterion, because the chance for intra-lineage polymorphisms was expected to be small if no inter-lineage polymorphisms were present. Inter-lineage polymorphic loci were analyzed further with a panel of eight EU1 isolates that belong to separate MG groups based on the study of Ivors and others (2006) or showed polymorphism in preliminary screening. Loci that were polymorphic in these selected isolates were analyzed in all isolates.

All *P. ramorum* isolates were evaluated for resistance to metalaxyl, based on Heungens and others (2006). Growth rate was determined between seven and 13 days post inoculation on the non-metalaxyl containing control plates.

Results and Discussion

The 411 isolates of *P. ramorum* included in this study were obtained from *Rhododendron* (90.5 percent of the plants sampled) or *Viburnum* plants (9.5 percent). At 61.6 percent of all nurseries where *P. ramorum* has been detected, epidemics were contained to a single year.

Our direct comparison using 80 isolates revealed more microsatellite-based diversity than AFLP-based diversity, which contrasts with the work of Ivors and others (2004). Microsatellite analysis was also more appropriate than AFLP, owing to its co-dominant nature and better reproducibility. After identifying a sufficient number of polymorphic markers, we opted for microsatellite analysis' technical simplicity and limited cost. However, identification of polymorphic microsatellite markers in a clonal population with low genetic diversity is difficult, even when the complete genome sequence is available. Many microsatellites are polymorphic between EU1 and NA1 isolates, but not within the EU1 population. Due to the limited overall genetic diversity within the EU1 lineage, screening of all 149 candidate primer pairs resulted in the identification of only two new primer pairs.

Microsatellite alleles were determined for the 411 Belgian isolates using the three primer pairs previously described (Ivors and others 2006) and the two new primer pairs. As one of the primer pairs amplifies two loci and the other amplifies three loci, a total of eight microsatellite loci were studied, seven of which were polymorphic. Based on these markers, 30 microsatellite genotypes (MG) were identified. In each year, the Belgian population was dominated by genotype EU1MG1 (68 percent of all isolates). Site-specific divergence was demonstrated at several nursery sites. The persistence of rare genotypes, and the intermittent absence of a unique genotype in a specific site, was observed. This indicates latent survival of *P. ramorum* and failure of eradication at some nurseries.

No indications of sexual or mitotic recombination were found. Selection for more metalaxyl-resistant isolates was correlated with a decrease of allelic richness. An upward trend in average growth rate and a significant reduction in growth rate variance indicated selection for faster-growing isolates.

Belgian isolates of *P. ramorum* were the subject of this work, owing to the geographical origin of the European A2 isolates. However, the microsatellite loci identified in this work are excellent markers for genotyping the general EU population of *P. ramorum*. The newly identified microsatellite loci are also polymorphic within the NA1 population and are therefore very useful for higher-resolution genotyping of this population.

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An Update on Microsatellite Genotype Information of *Phytophthora ramorum* in Washington State Nurseries[1]

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Abstract

Phytophthora ramorum was first detected in a Washington nursery in 2003 and has since been positively identified in 46 nurseries, three non-nursery water sites, and three landscape sites. Thirteen nurseries have tested positive for 2 consecutive years and four nurseries have been positive for 3 consecutive years, despite the completion of the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS) Confirmed Nursery Protocol (CNP), which is intended to eradicate this pathogen from positive nurseries. Trace-back inspections to wholesale nurseries have failed to confirm the origin of infested plant material at some nurseries and thus it is unclear if the pathogen is being reintroduced into these repeat nurseries each year or if it is persisting at the nurseries from one year to the next.

In an effort to better understand the population structure of *P. ramorum* in Washington, 328 isolates or DNA samples were collected in cooperation with the Washington State Department of Agriculture (WSDA) over 5 years (2005 to 2009) from 30 Washington nurseries, three non-nursery water sites, and three landscape sites, and genotyped using eight previously described microsatellite markers.

All three previously described lineages (EU1, NA1, and NA2) were detected in each of the 5 years. In this population, the EU1 lineage is represented by one genotype, the NA2 lineage is represented by one genotype, and the NA1 lineage is represented by 45 genotypes. The NA1 lineage was the most common, occurring in 25 nurseries, three non-nursery water sites, and one landscape site. The NA2 lineage was detected at 11 nurseries and one non-nursery water site, while the EU1 lineage was detected at seven nurseries and two landscape sites. The occurrence of the EU1 lineage in Washington has increased in frequency over the past 4 years while the overall number of sites and isolates has declined. At one nursery in 2007, the NA1 and EU1 lineages were isolated from different branches on the same rhododendron plant and at a different nursery in 2008 in the same soil bait. Although no genotype detected to date possesses a hybrid of alleles from both the European (EU1) and North American (NA1 and NA2) lineages, the combined presence of these lineages poses an increased risk to Washington because of the potential for sexual recombination.

The CNP appears to be effective in eradicating the pathogen from infested nurseries in some instances. Twenty-nine of the 45 positive nurseries were negative for *P. ramorum* in the year following completion of the CNP. On the other hand, 17 nurseries were positive for 2 or more years in a row. At only two repeat positive nurseries did a new lineage appear in the second

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positive year that was not present in the nursery during the first positive year; however, our genotype sample sizes are small. Thus it is unclear if inoculum was persisting from year to year in these nurseries or if the same genotypes were reintroduced in subsequent years.

Acknowledgments

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Population Genetic Analysis Reveals Ancient Evolution and Recent Migration of *P. ramorum*[1]

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Abstract

Phytophthora ramorum populations in North America and Europe are comprised of three clonal lineages based on several different genetic marker systems (Ivors and others 2006, Martin 2008). Whether these lineages are ancient or a recent artifact of introduction has been unclear. We analyzed DNA sequence variation at five nuclear loci in order to better understand the evolutionary history of the three lineages and the relationships among them (Goss and others 2009). We did not see differences among isolates within each lineage, therefore the observed genetic divergence among lineages precedes introduction. Analysis with coalescent-based methods revealed that the lineages have been diverged for an evolutionarily significant period of time, roughly 165,000 to 500,000 years. Genes contained signatures of historical recombination between the lineages, indicating that ancestors of the *P. ramorum* lineages reproduced sexually. The large genetic differences among lineages suggest that they were not introduced from a single interbreeding population. Instead, the three lineages likely originated from three different geographic locations such that they evolved in isolation from each other prior to introduction to North America and Europe.

Within the *P. ramorum* lineages, highly variable microsatellite markers have proved useful for examining population structure (Ivors and others 2006, Prospero and others 2007, Mascheretti and others 2008, Prospero and others 2009). *P. ramorum* has been moved via the nursery trade from source populations on the West Coast to locations across the U.S. (Frankel 2008). We investigated whether we could infer *P. ramorum* migration patterns in the U.S. nursery trade using existing microsatellites markers. We genotyped 279 isolates collected from infested nurseries in 19 states between 2004 and 2007 (Goss and others, unpublished data). This resulted in 53 multilocus genotypes in 228 NA1 isolates, 2 multilocus genotypes in 17 NA2 isolates, and 2 multilocus genotypes in 34 EU1 isolates. Our analysis focused on isolates in the NA1 lineage because of the limited distributions and low genetic diversities of the EU1 and NA2 clonal lineages in U.S. nurseries. A single NA1 genotype was shared among the majority of states and isolates clustered into two genetic groups, one mainly containing isolates from Connecticut, Oregon, and Washington and the other isolates largely from California and the remaining states. From this pattern we inferred two predominant eastward migration routes for NA1 individuals originating either in California or the Pacific Northwest. This is consistent with U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS) trace-forward and trace-back investigations, which indicate large shipments to 39 states from California and smaller shipments from Oregon in

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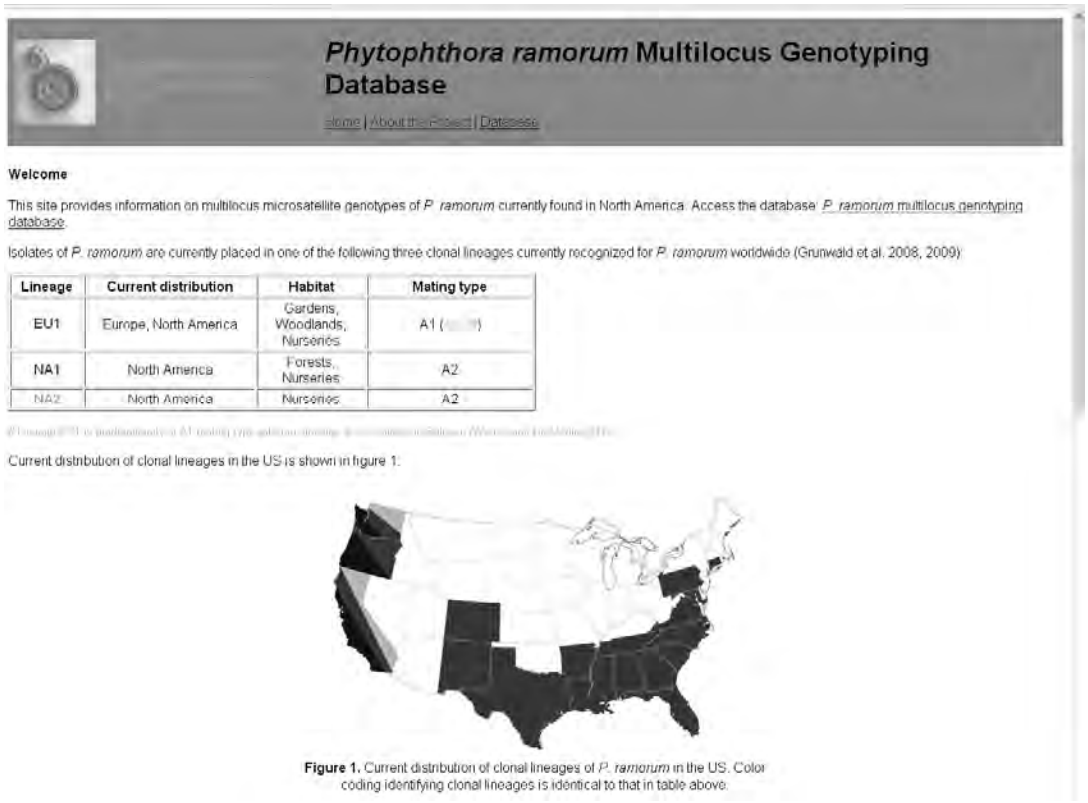
2004. Genotyping of North American nursery isolates is ongoing. The clonal lineage of each *P. ramorum* isolate genotyped is posted to a public website as soon as permission is obtained from the providing institution or agency (fig. 1).



Phytophthora ramorum Multilocus Genotyping Database

Welcome

This site provides information on multilocus microsatellite genotypes of *P. ramorum* currently found in North America. Access the database: *P. ramorum* multilocus genotyping database.

Isolates of *P. ramorum* are currently placed in one of the following three clonal lineages currently recognized for *P. ramorum* worldwide (Grunwald et al. 2008, 2009)

Lineage	Current distribution	Habitat	Mating type
EU1	Europe, North America	Gardens, Woodlands, Nurseries	A1 ( )
NA1	North America	Forests, Nurseries	A2
NA2	North America	Nurseries	A2

Current distribution of clonal lineages in the US is shown in figure 1:

Figure 1. Current distribution of clonal lineages of *P. ramorum* in the US. Color coding identifying clonal lineages is identical to that in table above.

Figure 1—*P. ramorum* multilocus genotyping database webpage (http://oregonstate.edu/~grunwaln/index.htm).

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Predicting Risk

Predicting the Spread of Sudden Oak Death in California (2010-2030): Epidemic Outcomes Under No Control[1]

Ross K. Meentemeyer,[2] Nik Cunniffe,[3] Alex Cook,[3] David M. Rizzo,[4] and Chris A. Gilligan[3]

Abstract

Landscape- to regional-scale models of plant epidemics are direly needed to predict large-scale impacts of disease and assess practicable options for control. While landscape heterogeneity is recognized as a major driver of disease dynamics, epidemiological models are rarely applied to realistic landscape conditions due to computational and data limitations. Here we describe a stochastic susceptible-infectious epidemic model, applied to temporally and spatially heterogeneous landscape parameters, to predict the spread of the invasive forest pathogen *Phytophthora ramorum*, the cause of sudden oak death (SOD), in California (1990 to 2030). Three epidemiological processes (production of inoculum, dispersal, and infection) are modeled on a weekly time step across a 250 m by 250 m lattice composed of variable susceptible and infected host units. We describe how field, lab, and geospatial data were combined to parameterize and map the key system variables affecting transmission of *P. ramorum*, including weather conditions, host infectiousness and availability, and a Markov Chain Monte Carlo estimated dispersal kernel. Replicated 1000 times to examine stochastic variability in epidemic outcomes, model predictions have a high degree of correspondence with 784 field plot observations that were collected across the pathogen's potential geographic range to validate model performance. Results show that most disease spread occurs via local dispersal (<250 m), but infrequent long-distance dispersal events can substantially accelerate epidemic spread in regions with large amounts of highly suitable habitat. While the epidemic is already widely distributed, we predict that, under no control, epidemic spread will increase 10-fold by 2030, with most infection concentrated along the north coast between the San Francisco Bay Area and Oregon. Moreover, wetter than normal weather conditions associated with possible climate change between 2010 and 2030 would double the rate of this spread. However, infrequent long-distance dispersal of inoculum to susceptible host vegetation in the Sierra Nevada and southwestern California ecoregions typically leads to little secondary disease spread in these regions due to low landscape connectivity of hosts and less suitable weather conditions for inoculum production and infection. This research illustrates how stochastic epidemiological models can be applied to realistic geographies and be used to gain a predictive understanding of SOD disease dynamics at landscape- to regional-scales.

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Strategies for Control of Sudden Oak Death in Humboldt County—Informed Guidance Based on a Parameterized Epidemiological Model[1]

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Introduction

The outbreak of sudden oak death (SOD) in Humboldt County, California is geographically isolated from the wider epidemic in central California. This situation offers an opportunity for containment, but also poses a danger of massive spread of *P. ramorum* (the pathogen that causes SOD) through the vast forest stretch that extends from Redway, southern Humboldt County, to Curry County, Oregon, due to favorable host and environmental conditions. There is a consensus on the need to implement a systematic strategy to manage *P. ramorum* in Humboldt County in order to reduce the local impacts (ecological, social, and economic) of the disease and to prevent further northward spread of the pathogen. While lessons can be learned from the extensive control efforts that have been implemented in Curry County, there is uncertainty as to which strategy to employ and as to what level of control is attainable in Humboldt County given the current size of the focus, limited resources available, and range of public opinion with regard to different options. In order to guide strategic planning, we forecast the epidemiological feasibility and risk of failure for a set of control scenarios. We do this for a period of 5 to 10 years using a mathematical model that represents the stochastic dispersal and transmission of *P. ramorum* on the local host landscape. The model is parameterized via Bayesian MCMC estimation of pathogen spread from aerial survey data. We consider control strategies and combinations thereof, starting in 2009 (together with scenarios to explore "what if control had been started earlier"). Four main control strategies are considered: (1) removal of inoculum in the area of the disease focus, (2) removal of inoculum away from the disease focus and ahead of symptoms, (3) aerial spraying with Agri-Fos® on a large scale, and (4) a host-free zone near to the van Duzen River. The results suggest that the large size of the Redway focus and potential for long-distance dispersal of *P. ramorum* pose considerable challenges to containment, but that local control in this focus or early containment of new, smaller foci are attainable. The insight provided by this study is relevant to other regions of California as effective control measures are probably optimally designed at a regional scale.

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Humboldt Epidemic

While the first cases of *P. ramorum* occurred in central California in the mid 1990s (Rizzo and others 2005), the first reports of *P. ramorum* infection in Humboldt County date from 2002. Since then the affected area in Humboldt County has grown at an increasing rate with significant mortality among tanoak (*Lithocarpus densiflorus*) and oak (*Quercus*) species over an estimated thousands of acres. The spread of disease has been predominantly northward of the initial focus near Redway and Garberville, probably due to prevailing winds. So far, only moderate, localized control measures have been applied in the county with evidence that these might have had an impact at the scale of the treated individuals and plots (Valachovic and others 2005, 2008).

Epidemiological Model and Control Scenarios

We have developed a probabilistic, spatially-explicit metapopulation model for the transmission dynamics of *P. ramorum* in the local host landscape; the main outcomes are risk maps of the probability of infection. The model's resolution is a 250 m by 250 m cell representing a mixed-host stand. Each cell's host composition, susceptibility, and infectivity are determined by the CALVEG GIS database (Meentemeyer and others 2004). At each time, each cell can be in one of the following four classes: susceptible; infected and asymptomatic (or cryptic); infected and symptomatic, and therefore detectable; or removed if host culling is in operation. Removed cells can be re-colonized via host re-sprouting or re-invasion. Infected cells can transmit inoculum to susceptible cells according to a dispersal-kernel function of relative distance. The model was parameterized using tanoak mortality data from aerial surveys in the Redway area between 2004 and 2007. We applied Bayesian Markov chain Monte Carlo estimation to infer the time and location of the index case (near Redway) and the rate and spatial scale of transmission, and to choose among candidate dispersal kernels (a long-tailed power-law fitted the data considerably better than a negative-exponential). We considered the following alternative control strategies:

1. Monitoring in a pre-determined area about once per year and removal of inoculum in detected symptomatic stands comprising host culling or herbicide treatment and pile burning (Valachovic and others 2008).
2. Agri-Fos® aerial spraying applied on a large scale (Kanaskie and others 2009) to provide temporary protection of hosts (for example, tanoak) and prevent northern spread. Treatment is applied in a strategy mixing of inoculum removal "at the origin" and, with the same frequency, spraying "ahead of symptoms" in low-density population areas.
3. A host-free "barrier" (Valachovic 2005) located near the Van Duzen River (Phillip Cannon, U.S. Department of Agriculture, Forest Service, Pacific Southwest Region, personal communication) to prevent northern spread.

We concentrated on a region approximately 85 km long from south to north containing the initial focus nearer the southern edge. For implementation of control and definition of northern invasion, we divided this region into three areas: **Area 1** (containing the focus, for "control at the origin"), **Area 2** (north of the focus and predicted to contain little disease if any in 2009, for "control ahead of symptoms"), and **Area 3** (predicted not to be infested in 2009 and to be protected from invasion). Areas 1 and 2 have the same size. The "barrier," located at the northern edge of Area

2, extends from east to west, is 5 km wide from north to south, and is hypothetically managed such that it remains host free.

Predicted Natural Spread

The epidemic front (defined as the region where the probability of invasion ranges between 95 percent and 5 percent) is forecast to advance northward at a speed of 7 to 8 km/year (but more slowly before 2006 when the focus was small at the scale of a unit cell). In 2009, the epidemic front is predicted to be between 25 and 35 km north of Redway (near the estimated initial focus), between Miranda and the Van Duzen River. These predictions rely on data that include 2 years with high spring rainfall and favorable spread, so it is possible that future estimates including data from 2008 and later would yield a slower rate of spread, although recent years have coincided with the La Niña weather cycle and the (warmer and wetter) El Niño cycle is expected to resume soon. The results therefore suggest a fairly rapid northern spread of *P. ramorum* in the medium and long term in the absence of large impact interventions.

Predicted Impact of Control Strategies Starting in 2009

Sustained removal of inoculum on a smaller scale than the size of the focus is effective locally (as indicated by a drop in the local **basic reproductive number** from > 10 to < 1 per infected host), but fails to contain northern spread due to continuing cryptic infection. Sustained removal on a larger scale than the size of the focus – either by increasing the control area or through early monitoring and rapid treatment – can provide local control and contain growth of the focus for several years. Agri-Fos® large-scale spraying can protect effectively and contain further spread for many years (depending on coverage and efficacy) if applied early and repeatedly ahead of the epidemic front. A 5 km-wide host-free barrier is ineffective in containing the Humboldt County focus as inoculum builds up and is able to jump over, at least under topographic and weather conditions analogous to those near Redway. Therefore, the currently large size of the Humboldt County focus and the long-distance dispersal of *P. ramorum* imply that, although it is in theory possible to eliminate the focus, the scale, nature of the treatment, and coordination needed to do so would make it unfeasible. However, the model suggests it is possible to control new, small foci through early detection, culling, and protection ahead of infection. This study relies on the currently limited epidemiological data and knowledge of the pathogen's biology. These findings are relevant to local control of *P. ramorum* in northern California and to areas of central California where the pathogen is at early stages of establishment or has not yet invaded.

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Mapping the Risk of Sudden Oak Death in Oregon: Prioritizing Locations for Early Detection and Eradication[1]

Tomáš Václavík,[2] Alan Kanaskie,[3] Ellen Goheen,[4] Janet Ohmann,[5] Everett Hansen,[6] and, Ross Meentemeyer[2]

Abstract

Phytophthora ramorum was first discovered in forests of southwestern Oregon in 2001. Despite intense eradication efforts, disease continues to spread from initially infested sites because of the late discovery of disease outbreaks and incomplete detection. Here we present two GIS predictive models of sudden oak death (SOD) establishment and spread risk that can be used to target monitoring and eradication activities in western Oregon. Model predictions were based on three primary parameters: weather and climate variability, host vegetation susceptibility and distribution, and dispersal (force of infection). First, a heuristic model using multi-criteria evaluation (MCE) method was developed to identify the areas at *potential* risk. We mapped and ranked host susceptibility using new geospatial vegetation data available from the U.S. Department of Agriculture, Forest Service (USDA FS)/Oregon State University (OSU) Landscape, Ecology, Modeling, Mapping, and Analysis project (LEMMA). Precipitation and temperature conditions derived from PRISM climate database were parameterized in accordance to their epidemiological importance in the SOD disease system. The final appraisal scores were calculated and summarized to represent a cumulative spread risk index, standardized into five risk categories from very low risk to very high risk. Second, we used the machine-learning method, maximum entropy (MAXENT) to predict the current distribution of SOD infections. Here, probability of infection was calibrated based on the correlation between 500 field observations of disease occurrence and several predictor variables including climate variability, host susceptibility and abundance, topographic variables, and a dispersal constraint. The dispersal constraint estimates the force of infection at all locations and thus predicts the actual or current distribution of the pathogen rather than its potential distribution. Numerous forests across the western region of Oregon appear to be susceptible to SOD. Areas at greatest risk of disease spread are concentrated in the southwest region of Oregon where the highest densities of susceptible host species exist, in particular tanoak (*Lithocarpus densiflorus*). These models provide a better picture of threatened forest resources across the state and are being actively used to prioritize early detection and eradication efforts.

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Introduction

Phytophthora ramorum was first discovered in forests of southwestern Oregon in 2001. In contrast to the pathogen's relatively wide distribution throughout coastal California, in Oregon, it occurs only in one small area in Curry County near the town of Brookings. Despite intense eradication efforts, consisting of clearcuts, burns, and herbicide applications, disease continues to spread from the initially infested sites. It is believed that the major reason why control activities have been partially effective is the late discovery of disease outbreaks and incomplete detection. Since field and aerial surveys are labor intensive and costly methods, predictive risk models can be effective alternatives for prioritizing areas for early detection and eradication treatments. Predictive risk models have been developed and used in California (for example, Meentemeyer and others 2004, 2008), but similar modeling has been limited in Oregon due to unavailable vegetation data. Using new data available from the U.S. Department of Agriculture, Forest Service (USDA FS)/Oregon State University (OSU) Landscape, Ecology, Modeling, Mapping, and Analysis project (LEMMA) we present two GIS predictive models of sudden oak death (SOD) potential spread risk and actual distribution that can be used to target monitoring and eradication activities throughout six forest ecoregions in western Oregon.

Methods

Model predictions were based on three primary parameters: weather and climate variability, host vegetation susceptibility and distribution, and force of infection. First, we developed a heuristic (rule-based) model using multi-criteria evaluation (MCE) method to identify the areas at potential risk of SOD establishment and spread. Following methods described in Meentemeyer and others (2004) for California, we mapped and ranked host susceptibility using new geospatial vegetation data from the LEMMA project. These vegetation data were developed based on extensive sample-based field inventories and combination of multivariate statistics and gradient nearest-neighbor (GNN) imputation resulting in maps of detailed vegetation composition and structure in forest and woodland areas (Ohmann and Gregory 2002). We compiled these vegetation data to create a host index calculated as a product of abundance scores and species spread scores. We followed the scoring scheme in Meentemeyer and others (2004), ranking each host's potential to produce inoculum and spread the disease to other hosts (table 1). However, we assigned tanoak, *Lithocarpus densiflorus*, (instead of myrtlewood, *Umbellularia californica*) the highest score because tanoak appears to play a more important epidemiological role in Oregon than myrtlewood.

Table 1—Spread scores of host species based on their potential to spread inoculum of *P. ramorum*

Hosts	Score
Arbutus menzeisii – Pacific madrone	1
Arctostaphylos spp. – pinemat manzanita	1
Frangula californica – California buckthorn	1
Frangula purshiana – Pursh's buckthorn	1
Lithocarpus densiflorus – tanoak	10
Lonicera hispidula – pink honeysuckle	1
Pseudotsuga menziesii – Douglas-fir	1
Quercus chrysolepis – canyon live oak	0
Quercus kelloggii – California black oak	0
Rhododendron sp.	5
Rubus spectabilis – salmonberry	1
Sequoia sempervirens – coast redwood	3
Umbellularia californica – myrtlewood (California bay laurel)	5
Vaccinium ovatum – evergreen huckleberry	1

In addition to vegetation parameters, precipitation and temperature conditions derived from PRISM climate database (Daly and others 2001) were parameterized in accordance to their epidemiological importance in the SOD disease system (table 2).

Table 2—Range of values and assigned scores (*R*), ranked 0 to 5 from least to most suitable for establishment and spread of *P. ramorum*

Score (Ranks)	Precipitation (mm)	Average maximum T (ºC)	Average minimum T (ºC)
5	> 125	18-22	-
4	100-125	17-18; 22-23	-
3	75-100	16-17; 23-24	-
2	50-75	15-16; 24-25	-
1	25-50	14-15; 25-26	> 0
0	<25	< 14; > 26	< 0

The final appraisal scores were calculated and summarized for the entire area to represent a cumulative spread risk index, standardized into five risk categories from very low risk to very high risk. Each parameter was assigned a score between 0 and 5, with 5 representing the conditions that are most suitable for establishment and spread of *P. ramorum*. Weights on a scale of 1 to 6 were assigned to each variable, based on their relative importance for disease spread (table 3). Using the weights and scores of the four parameters, the final spread risk (appraisal score) was computed for each grid cell by finding the sum of the product of each scored variable and its weight:

$$\overline{S} = \sum_i^n W_i R_{ij}$$

where $\overline{S}$ is the appraisal score (spread risk) for a grid cell, W_i is the weight of the *i*th predictor variable, and R_{ij} is the rank, or score, of the *j*th value of the *i*th variable.

Table 3—Importance weights (*W*) assigned to predictor variables, ranked 1 to 6 from lowest to highest importance for *P. ramorum* (according to Meentemeyer and others 2004)

Variable	Weight
Host species index	6
Precipitation	2
Maximum temperature	2
Minimum temperature	1

Second, we used maximum entropy (MAXENT) to predict the current distribution of SOD infections within the 2008 quarantine area designed by the Oregon Department of Agriculture. MAXENT is a machine-learning method that estimates distributions of organisms by finding the probability distribution of maximum entropy (in other words, the most uniform) given the constraint that the expected value of each environmental predictor under this estimated distribution matches the empirical average of sample locations (Phillips and others 2006). We calibrated the relative likelihood of infection based on the relationship between more than 500 field observations of disease occurrence in the period 2001 to 2007 and several predictor variables including climate variability, host susceptibility and abundance, topographical variables, and a dispersal pressure. The dispersal term, calculated as the cumulative inverse distance between each target plot and other sources of infection (Allouche and others 2008), estimates the relative force of infection at all locations and thus predicts the actual or current distribution of the pathogen rather than its potential distribution. We validated the accuracy of the model by comparing its predictions with independent field observations of disease outbreaks recorded in 2008 and calculating the area under the curve (AUC) of the receiver operating characteristic (ROC).

Results

The final risk map produced by the heuristic model shows a distinct geographical pattern of *P. ramorum* establishment and spread risk in western Oregon based on the influence of host species abundance and climate parameters (fig. 1). Although there are large areas with very low and low *P. ramorum* risk in the eastern part of the area due to low host availability and unfavorable climate conditions, numerous forests across the western region of Oregon appear to be susceptible. The forests at greatest risk of disease spread are concentrated in the southwest region of Oregon where the highest densities of susceptible host species exist, in particular tanoak. From the total area of 66,000 km^2 of forest with susceptible host vegetation, over 250 km^2 were identified as very high risk, 1,870 km^2 as high risk and 4,200 km^2 as moderate risk.

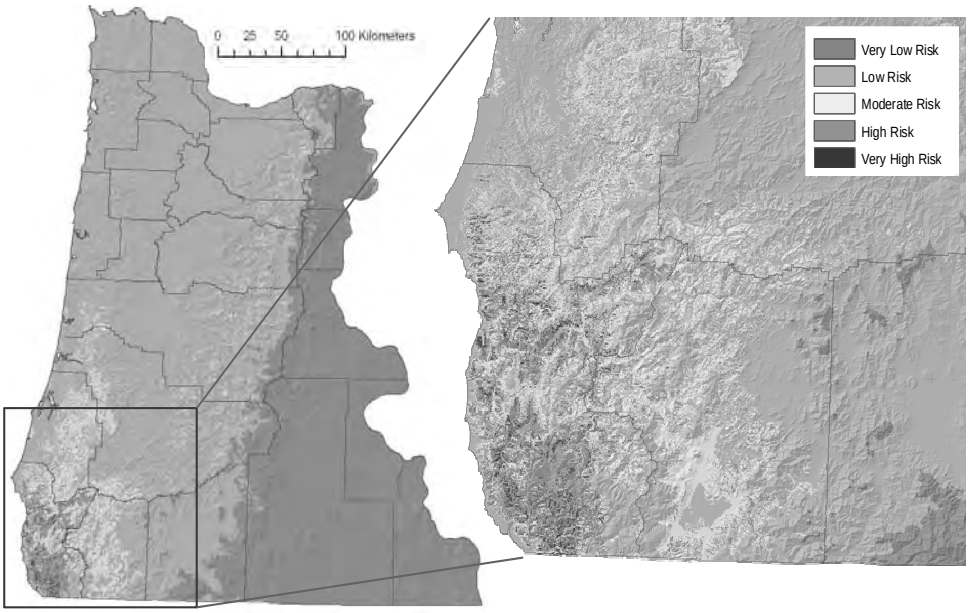

Figure 1—Predicted risk map of *P. ramorum* potential risk of establishment and spread in western Oregon.

The statistical model based on maximum entropy showed that likelihood of SOD is positively associated with temperature and precipitation, and negatively associated with elevation and potential solar radiation. Tanoak abundance was strongly associated with SOD presence, followed by evergreen huckleberry (*Vaccinium ovatum*), salmonberry (*Rubus spectabilis*), Douglas-fir (*Pseudotsuga menziesii*) and myrtlewood. Application of the MAXENT model to map each of these variables predicted the current distribution of SOD (fig. 2). The highest likelihood of disease is concentrated in the southwest portion of the quarantine area and along the North Fork of the Chetco River (fig. 2). When the predicted likelihood of pathogen's presence was validated with field data from 2008, AUC of the ROC statistic was 0.95, suggesting relatively high prediction accuracy.

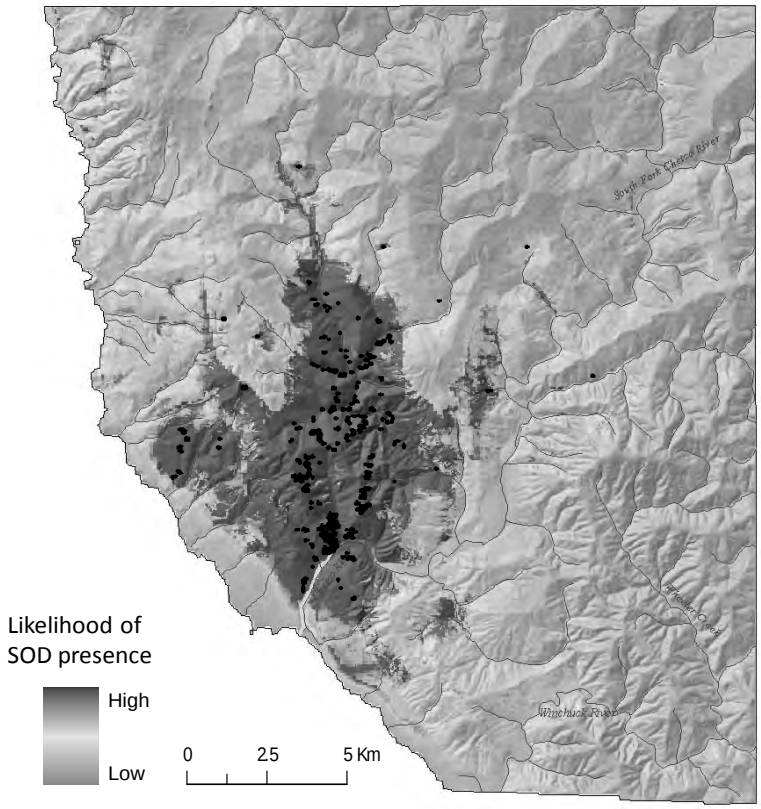

Figure 2—Predicted actual distribution of *P. ramorum* in 2008 quarantine area in southwest Oregon.

Conclusions

The two modeling approaches presented in this work build from risk modeling research previously applied in California (Meentemeyer and others 2004, 2008) and produce the first risk models of SOD specifically designed for Oregon. Although the area of current SOD infestation in Oregon is relatively small, there is more than 2,000 km^2 of forest at high and very high potential risk of disease establishment and spread. These predictive models provide a better picture of threatened forest resources across the state. As new infested sites are discovered, risk models will be updated and validated with new data, and used to prioritize locations for on-the-ground management and early detection surveys.

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Regulatory Considerations in Assessing the Potential for *Phytophthora ramorum* to Cause Environmental Impact to Ecozones Outside the West Coast "Fog Belt" in North America[1]

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Abstract

Sudden oak death (SOD) is a disease caused by *Phytophthora ramorum* that is characterized by lethal trunk lesions that affect tanoak (*Lithocarpus densiflorus*), and a few oak species, principally coast live oak (*Quercus agrifolia*). It was first observed in Marin County, California, in 1994, and now has been reported to have caused extensive tree mortality in the West Coast "fog belt" area that generally extends less than 30 km inland, from Monterey County, California, in the south, to Curry County, Oregon, in the north. While the plant communities vary somewhat, it is notable that the zone closely mirrors the distribution of the coast redwood (*Sequoia sempervirens*). Tanoak and California bay laurel (*Umbellularia californica*) are present throughout the zone. In California, infected bay laurel produce abundant *P. ramorum* sporangia which facilitate the spread of the pathogen, but in Oregon pathogen spread is primarily attributed to inoculum produced on tanoak foliage. Climate-host models generated to assist in early detection, survey, mitigation, and formulation of regulatory policy, have generally indicated that the west coast and the eastern conterminous United States (U.S.), especially the Appalachian Mountains, are at similar risk for infection. This risk area also extends up into coastal British Columbia, Canada. Indeed, regulatory surveillance and certification programs to detect *P. ramorum* on nursery plants moving in inter-state trade, both in the U.S. and Canada, have resulted in detections in many of these areas since 2002, suggesting the ability of the pathogen to survive and move within the nursery environment. However, no *P. ramorum* forest or wildland areas has been reported outside the current ecozone in California and Oregon, despite large shipments of host plants from known infested areas. Possible reasons for this, with consideration of when and how *P. ramorum* may have been introduced to North America, what is achievable through nursery certification, and the epidemiological uniqueness of the *P. ramorum* ecozone in the West Coast "fog belt", are discussed from a regulatory perspective.

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When and How *P. ramorum* was Introduced to North America

Microsatellite analyses of *Phytophthora ramorum* populations in California (Mascheretti and others 2008) have suggested that the most likely pathway for introduction of the pathogen into the state was through the importation of infected nursery plants. When this might have occurred is quite speculative, but working back from the time it might take for the pathogen to enter a nursery, establish, escape into the wild and be noticed, it has been estimated that it has been in the California nursery pathway since at least the mid-1980s (M. Garbelotto, Extension Specialist and Adjunct Professor, University of California, Berkeley, personal communication). This scenario would suggest that *P. ramorum* has now been present in California nurseries for over 25 years.

Regulatory Control Through Nursery Certification

Regulatory surveillance to prevent the interstate movement of *P. ramorum* in the nursery pathway began in 2002 with controls put on the infested counties in California and Oregon (USGAO 2006). In 2004, this regulated area was expanded to include all of California. By 2005, a Federal Order was issued that required that all nurseries in California, Oregon, and Washington that shipped host plant nursery stock interstate, be inspected and certified free of evidence of *P. ramorum* (USGPO 2007). It has been argued that this regulatory nursery certification program appeared to have been effective at minimizing the interstate dissemination of the pathogen (Suslow 2008). Inspection and survey data from 2004 to 2006 indicated that the number of infested nurseries dropped by more than 50 percent, from 110 nurseries to 50 (representing one to two percent of the nurseries inspected), respectively. However, despite this progress, positive nurseries have continued to be detected every year since.

Nursery certification faces a number of challenges. *P. ramorum* detection is predicated on observing, sampling, and testing symptomatic tissue. Plant infections with asymptomatic sporulation, as reported by Denman and others (2008), can occur and therefore be missed. *P. ramorum* also has the potential to be moved from a nursery in the potting media of asymptomatic non-hosts (Dart and Chastagner 2007).

Testing for certification is typically done in a two step process: first by conducting an enzyme-linked immuno-sorbent assay (ELISA) for the *Phytophthora* genus and then confirming the presence of *P. ramorum* by either polymerase chain reaction (PCR) or by culturing the organism (Bulluck and others 2006). The overall sensitivity of this process is limited by ELISA as it is less sensitive than the confirming tests, particularly as selection of the ELISA threshold is influenced by the need to minimize the number of false positives. Thus, a certain proportion of false negatives are inevitable. In addition, the resource constraints, imposed by the costs of sampling and testing, generally mean that no more than 40 samples per nursery are taken, which relative to the potential thousands of plants and millions of leaves in a typical nursery, is a small number.

The effects of all these challenges mean that *P. ramorum* has almost certainly been moving undetected in the North American nursery pathway for many years. The

potential for widespread dissemination through this pathway is illustrated by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS) investigation of a California nursery outside the quarantine counties in 2004. In this instance, it was determined that the nursery had shipped potentially infected camellias to over 1,200 establishments in 39 states. *P. ramorum* was subsequently detected at 175 of these sites in 22 states (USGPO 2007).

Epidemiological Uniqueness of the SOD Ecozone in Relation to Regulatory Policy

In considering why *P. ramorum* has not been reported to have caused tree mortality in North America, outside its current coastal redwood forest ecozone in the West Coast (Rizzo and others 2005), one possibility is that it has not had the opportunity to jump from infected nursery plants to vulnerable wildlands, as it apparently did in California. However, prior to 2005, when regulatory restrictions on its interstate movement started, there were likely 20 years when there was extensive and unimpeded movement of host nursery stock from the West Coast to eastern areas, including those considered at highest risk (Koch and Smith 2008, Magarey and others 2008), thus suggesting opportunities, comparable to those that apparently occurred in California, for *P. ramorum* to be introduced to these areas. Indeed, since the start of national surveillance in the U.S. in 2004, documented detections have repeatedly occurred in various states in addition to California, Oregon, and Washington (http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/updates/2008-annual-update.pdf).

In California, evidence of SOD was present in the early 1990's and by the mid 2000's much of the current range had been filled in. The apparent rapidity with which this spread occurred, combined with the empirical evidence for movement through the North American nursery pathway, would suggest that the conditions in the high-risk areas in the east are probably less conducive to tree mortality than those on the West Coast.

The coastal redwood forest is a unique ecozone that is not duplicated elsewhere in North America. The epidemiology there of *P. ramorum* suggests a critical need for extensive sporangia production, principally on California bay laurel, in close proximity (<10 m) to tanoak and oak trees, in order for formation of bole cankers to occur in a conducive environment (Swiecki and Bernhardt 2007). Thus, the apparent requirement for these particular conditions may place very real constraints on the potential for *P. ramorum* to cause bleeding cankers on bole hosts elsewhere. It is noteworthy though that bole cankers from *P. ramorum* can occur in other ecozones as approximately 40 trees with this disease have been reported from the United Kingdom and the Netherlands (Webber 2008). However, in contrast to the situation in California, these infections have been very limited and have been principally associated with diseased rhododendrons growing in close physical proximity to the infected trees. The differences in both scope and impact between the North American and European situations illustrates how differences in host plant communities and environmental conditions can profoundly affect the impact of this pathogen.

As noted by Hansen (2008), one of the breakthroughs in *P. ramorum* research has been the realization that the pathogen causes very different diseases on its many

different hosts (Davidson and others 2003). These diseases range from minor foliar lesions to stem cankers and tree death. Despite that, the sudden oak death moniker is commonly applied indiscriminately to all infections of *P. ramorum*. For regulators, though, these disease differences are important as the International Plant Protection Convention (IPPC) obliges the phytosanitary measures of contracting parties be "limited to what is necessary to protect plant health" and that these measures should "represent the least restrictive available" (FAO 2006a). Thus, if there is potential in an area for a quarantine pest to cause tree mortality, strong regulatory measures, commensurate with the potential impact, can be justified to protect that area. Conversely, if there is negligible risk of observable impact, strong measures may not be justified.

The regulatory history of *P. ramorum* has been cited in arguing for a more precautionary approach to trade in plants-for-planting (Brazier 2008). Indeed, where appropriate, a case can be made for using precautionary measures, such as post-entry quarantine, when new plant species are imported from new origins, to reduce the risk of introduction of new and damaging pathogens. However, whether current policies to prevent economic and environmental impact from the introduction and/or movement of *P. ramorum* in North America and Europe continue to be needed, or are effective, is another question. It can be argued that in contrast to the situation that regulators faced in 2001, when this alarming new pathogen was reported, the subsequent accumulation of scientific and empirical evidence today has now greatly reduced uncertainty in the epidemiology of *P. ramorum*, which should now allow for a less precautionary approach in assessing risk and in setting regulatory policy.

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Development of a Pest Risk Analysis for *Phytophthora ramorum* for the European Union; the Key Deliverable from the EU-Funded Project RAPRA[1]

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Abstract

Pest Risk Analysis (PRA) is an internationally recognized, structured process of determining whether plant pests and pathogens that are absent from a country or area could enter, establish, and cause an economic or environmental risk that is deemed unacceptable. PRA is also used to help identify phytosanitary measures to reduce risks to an acceptable level. United Kingdom (U.K.) PRAs for *Phytophthora ramorum* have been produced and developed since 2000, starting with the unknown *Phytophthora* causing sudden oak death in California, United States (U.S.). Other European Union (EU) Member States (MS) have also assessed the risk. As a result of the PRAs, *P. ramorum* was identified as posing a risk to the environment, private and managed gardens, and woodlands as well as to the ornamental plant trade in the U.K./EU/EPPO region. The prediction that heathland habitats were at risk (based upon host range testing and climatic matching) has now been proven by recent findings of *P. ramorum* (and *P. kernoviae*) on bilberry (*Vaccinium myrtillus*) in heathland in the U.K. Supported by the PRAs, emergency legislation was implemented in the U.K. and subsequently in the EU, allowing action to be taken against *P. ramorum* whenever it was found. National research projects were commissioned in the U.K. and elsewhere to help fill the gaps in the PRAs; these were inevitable, given the lack of knowledge on this newly identified species.

A major, multi-faceted EU-funded research project 'RAPRA' (Risk Analysis for *Phytophthora ramorum*) commenced in 2004 (http://rapra.csl.gov.uk/). The overall aim was to develop a European-wide PRA for *P. ramorum* for the 27 MS of the EU; this was to be based on the project's research findings as well as those emerging in the scientific literature. The project documented the increasing host range and geographical distribution of *P. ramorum*, including the distribution of mating types; helped determine the potential for sexual recombination; and investigated the potential future host range and aspects of epidemiology related to establishment risk. Refinement of the risk of establishment within the PRA accounted for these findings as well as the results of climatic matching and mapping using several methods. Economic impacts were difficult to assess since currently in the EU *P. ramorum* affects the commercial plant trade, the natural environment, and historic gardens - with secondary effects on tourism, particularly for southwest England. Commercial forestry is not yet affected, but may be at risk. Recommendations for future management of the risk of entry (for pathways identified from an earlier European and Mediterranean Plant Protection Organization [EPPO] PRA for *P. lateralis* and by evaluating trade data and existing phytosanitary legislation) were made. Potential measures for managing outbreaks in the EU that were proposed in the PRA were based on a review of existing measures as well as experimental results for disease management. This PRA is the key output from RAPRA and is

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being used in 2009 to review existing EU phytosanitary policy for *P. ramorum*. This paper summarizes some of the processes for determining the risk of entry, establishment and impacts posed by *P. ramorum* to the EU, and highlights areas of uncertainty. Full details are available in the PRA at http://rapra.csl.gov.uk/.

Introduction

Phytophthora ramorum, first described in 2001, is considered to be exotic to Europe and North America and is thought to have been relatively recently introduced separately to both continents from an unknown area, or areas, of origin, speculated to be somewhere in Asia. Prior to being formally named and described, the pathogen was first observed infecting rhododendron and viburnum in Germany and rhododendron in the Netherlands, since at least 1993. At around the same time, increased mortality of tanoak and oaks (*Lithocarpus densiflorus* and *Quercus* species) was observed in California, U.S. and this was described as 'sudden oak death' (SOD); the causal agent was identified as a new and unnamed *Phytophthora* species in 2000. The first formal pest risk assessment in Europe for the U.S. 'unknown' *Phytophthora* was produced by the U.K. in September 2000 (Brasier 2000). No specific phytosanitary measures were identified as the assessment was undertaken using the European and Mediterranean Plant Protection Organization (EPPO) risk assessment scheme, which pre-dated the EPPO Pest Risk Analysis (PRA) scheme, thus not including the third stage of PRA risk management. The conclusion of the assessor was that the U.S. *Phytophthora* had potential to establish in the U.K., possibly entering on nursery stock, and that it posed a significant risk to (at least) U.K. native and exotic oaks. By January 2001, the *Phytophthora* causing SOD in California and the *Phytophthora* which had been isolated from shrubs in the Netherlands and Germany were considered to be the same species. EPPO, the Regional Plant Protection Organization for many European and Mediterranean countries including the EU, added the pathogen to their Alert List (an early warning, without a full PRA) in January 2001. A second PRA was produced by the U.K., connecting the U.S. and EU findings. An EPPO-style Datasheet was also produced but was never published, although it was updated with each subsequent revision of the U.K. PRA described below. The revised PRA highlighted the risks to the U.K., EU, and EPPO region; identified uncertainties and research needs; and recommended surveys in the EU/EPPO region to determine the pathogen's distribution. It also advised that phytosanitary measures should be considered, such as controls on imports of known susceptible hosts and their products into and within the EU/EPPO region from areas/countries where the pathogen had been found, to try and prevent further entry. It was recommended that the pathogen should continue to be on the EPPO Alert List and that consideration should be given to making it an EU/EPPO quarantine pest. As a result of the PRA, in the summer of 2001, the Department for Environment, Food and Rural Affairs (DEFRA) Plant Health and Seeds Inspectors (PHSI) for England and Wales and the Dutch Plant Protection Service commenced limited surveys for the as yet unnamed *Phytophthora*.

After the pathogen was formally named as *P. ramorum* in October 2001, a third formal U.K. PRA was published in January 2002 for this newly identified species. In February 2002, as a result of the ongoing survey work, DEFRA detected *P. ramorum* on a symptomatic *Viburnum tinus* plant from a garden center in southern England. This was the first U.K. record of *P. ramorum.*

Following on from the PRA work, U.K. (England) legislation aimed at *P. ramorum* was enacted in May 2002. This was somewhat earlier than the European Commission (EC) legislation which came into force in September 2002, based largely on actions taken by the U.K. and the Netherlands. The U.K. (England) legislation was revoked and replaced in November 2002, reflecting the first EC requirements. The EC legislation broadened controls on imports of susceptible material and had requirements for controls on movement of susceptible plants within the EU, as well as controls on outbreaks, and a requirement for EU Member States (MS) to conduct surveys to be reported back to the EC by December 2003. One other European PRA (a report of a PRA) was prepared by the Netherlands in October 2002 to ensure that phytosanitary measures arising from the new EC legislation to be taken in that country were technically justified.

Accounting for ongoing research results (the U.K. research program commenced in 2002), literature, and findings of *P. ramorum* in the EU and North America, the U.K. PRA was updated again and was published in March 2003. It was revised further and published again in October 2003. This last revision pre-dated the first tree findings in the U.K. and the Netherlands in late October 2003. In 2004, a full update of the Datasheet was prepared and a draft PRA begun, but prior to completion, the first U.K. findings of the new pathogen *P. kernoviae* (in October 2003), followed by the expansion in its host range over the following year, led to changing priorities, and the PRA work for *P. ramorum* was put on hold.

More informal assessments of risk have also been made by other countries for Europe and for specific regions, for example, for the Mediterranean and for Italy. The U.S., Canada, and Australia have also prepared PRAs.

In 2007, a full update of the U.K. Datasheet (Sansford and Woodhall 2007), accounting for the results of the U.K. research program; key aspects of the EU and U.S. research program, including elements of the RAPRA (Risk Analysis for *P. ramorum*) project; and EU and North American survey findings was prepared. This was done to re-examine the risks to the U.K. and to suggest risk management options in preparation for the 2008 DEFRA public consultation for the future management of *P. ramorum* (and *P. kernoviae*) in Great Britain (GB). The U.K. Datasheet was used as one of several resources for preparing the RAPRA PRA.

The RAPRA project commenced in January 2004. Its ultimate aim was to produce a PRA for the EU assessing the risks posed by *P. ramorum* to European trees, woodland ecosystems, and other environmentally important habitats (for example, heathlands), as well as ornamental plants in the nursery trade and public gardens. This PRA was intended to be used to support a review of EU policy for *P. ramorum*. Meanwhile, EC legislation for the pathogen was amended in April 2004 and March 2007, accounting for changes in host range, survey results, and assessed risk. Current measures (pending review) still require official surveys to be reported back to the EC at the end of the year, and broadly-speaking, import and internal movement controls of rhododendron, viburnum, and camellia (the three most commonly affected traded genera in the EU) with statutory action to be taken on findings.

The PRA Process; Dealing with Uncertainty Through Research

The PRA process is a structured and logical approach to assessing the risks of entry, establishment and potential impact of plant pests and pathogens (collectively known in the PRA world as 'pests'), and, if necessary, identifying risk management options to reduce the assessed level of risk to an acceptable level. In Europe, PRAs are undertaken for individual organisms. Before a PRA can be undertaken, it must be clearly identifiable as unique (at least to species level - even if they are yet to be formally named). Thus, for newly identified diseases/pathogens, once Koch's Postulates have been completed, it is possible to conduct PRAs on previously unknown *Phytophthora* spp. such as those undertaken for *P. ramorum* (initially *Phytophthora* sp.) and *P. kernoviae* (initially *Phytophthora* taxon C. sp. nov.).

Pest risk analysts use national or regional decision-support schemes which are based upon the framework of the Food and Agriculture Organization (FAO) International Standard for Pest Risk Analysis (FAO 2004). One such scheme is the EPPO Standard 'Guidelines on Pest Risk Analysis: Decision-support scheme for quarantine pests' (EPPO 2007) – the 'EPPO scheme'.

During the PRA process, pest risk analysts inevitably identify uncertainties, especially for new species, and, at least in the U.K., we try to suggest further work that could be undertaken to address these and improve the PRA. The main areas under which these uncertainties are identified are in taxonomy, geographic distribution, hosts and potential hosts, pathways of entry, risk of establishment and spread, potential impact, and risk management – including non-statutory control. Suggestions for further work could be, for example, a relatively simple survey to help better determine the geographic distribution of an organism or, alternatively, a list of topics for which single or multi-faceted research projects could be commissioned to generate data to help fill the gaps in our knowledge. This allows us to revise our existing PRAs, update the assessment of risk, and, where appropriate, revise the suggested risk management options for consideration by national or EU policy makers.

The RAPRA Project

The RAPRA project was the second in a series of three EC-commissioned and part-funded projects, aimed at developing multi-faceted aspects of existing national PRAs for specific plant pathogens that had been assessed and predicted to pose a major threat to sectors of EU agriculture, horticulture, forestry, and/or the environment, and for which an EU-wide consensus on the risk was required.

The first project of this type was 'Karnal bunt risks' (http://karnalpublic.pestrisk.net/), conducted under the EU Fifth Framework program, which developed a PRA for *Tilletia indica*, the cause of Karnal bunt of wheat. The structure of this project became a model for two further projects under the EU Sixth Framework program: 'RAPRA' (http://rapra.csl.gov.uk/) and, subsequently, 'Pepeira' (http://www.pepeira.wur.nl/), to develop PRAs for *P. ramorum* and for Pepino mosaic virus (a damaging pathogen of tomato), respectively.

RAPRA was a project of 39 months duration, supported by >50 percent funding from the EC with nine partner institutes based in France, Germany, the Netherlands, Spain, the U.K., and the U.S., with three observer institutes in Belgium and Italy. The project was coordinated by the U.K. (Dr Joan Webber, Forest Research).

RAPRA was split into eight work packages (WP) which addressed eight scientific objectives. In numerical order of WP and objective, these were to

1. Collate and publish available information on the extent of entry and distribution of *P. ramorum* in the EU and Europe.
2. Establish the level of susceptibility (to both European and American isolates) of tree and non-tree species of significant environmental and economic value to the EU.
3. Quantify the sporulation, germination, infection, incubation period, latency, survival, and dispersal components of the epidemiology of European and American isolates of *P. ramorum*.
4. Establish the potential for mating between *P. ramorum* (predominantly A1 mating type) found in Europe and *P. ramorum* (predominantly A2 mating type) present in the U.S.
5. Evaluate the likely environmental and socio-economic impact of *P. ramorum* in the EU.
6. Evaluate at least three existing and at least two new chemical active ingredients for the control of *P. ramorum* in ornamentals.
7. Define outbreak scenarios, evaluate existing strategies for eradication and containment, and produce technical guidelines for management plans for dealing with *P. ramorum* in Europe while minimizing the need to disrupt free trade.
8. Develop, refine, and publish a European PRA for *P. ramorum* and provide information to underpin and advise EU plant health policy and legislation.

The newly generated experimental and economic data arising from WPs one to seven were incorporated into Deliverable Reports. These reports, along with a review of the literature up until November 2008, and earlier PRAs (most recently the 2007 U.K Datasheet), were used in WP8 to provide an assessment of the risk from North American and European isolates to the EU, and to determine risk management options.

The PRA was prepared using the EPPO scheme. The area for which the risk was assessed (the PRA area) was the 27 MS of the EU (fig. 1).

Figure 1—The PRA area: the European Union 27 MS. Source: http://encarta.msn.com/media_941538636_761579567_-1_1/map_of_the_european_union.html.

A summary of the process undertaken during the construction of the PRA, the data that were used, and the main findings (excluding risk management) and uncertainties are given below.

Assessment of the Risk of (Further) Entry of *P. ramorum* to the EU

The first step in determining the risk of further entry of *P. ramorum* into the EU was an assessment of the current geographic distribution documented in the RAPRA database (http://rapra.csl.gov.uk/objectives/wp1/distribution.cfm) of 'distribution and potential for spread of *P. ramorum* in Europe' (WP1). This assessment also took account of the known distribution of the three currently known distinct genetic lineages (EU1, NA1, and NA2) and mating types (A1 and A2). We also used the EU MS survey results from 2002 onwards, and published reports from the literature. The second step was to determine the main pathways of entry and the commodity types for which were selected as those identified in a 2006 EPPO PRA for *P. lateralis* (http://www.eppo.org/QUARANTINE/Pest_Risk_Analysis/PRA_documents.htm). Lists of known and potential hosts on which *P. ramorum* could enter were generated from the WP1 natural host database and WP2 (experimentally susceptible hosts are listed in the WP1 database), plus published literature and unpublished results. Trade data for 2003 to 2007 were obtained from the Eurostat Comext database and supplied by DEFRA. The EC phytosanitary legislation was also reviewed.

Current Geographic Distribution of *P. ramorum* – Potential Sources of Entry

A short summary of the distribution of *P. ramorum*, which identifies the likely sources of further entry to the EU, is given below.

U.S.–

Phytophthora ramorum is present in the wild in California and Oregon. The first nursery findings were made in California in 2001, with subsequent finds in Oregon and Washington State. In 2004, trade from California and Oregon led to the detection of *P. ramorum* in nurseries in 22 U.S. states; all were subject to eradication. Additional nursery finds have been made in the U.S. in subsequent years.

Canada–

Phytophthora ramorum has been reported (under eradication) in British Columbia, Canada in a few nurseries (first finding in 2003) and some related residential plantings.

Europe–

Phytophthora ramorum is found in 19 EU countries, but under official control in: Belgium, Czech Republic (eradicated nursery finding), Denmark, Estonia, Finland, France, Germany, Ireland, Italy, Latvia, Lithuania, Luxembourg, the Netherlands, Poland, Portugal, Slovenia, Spain (including Mallorca), Sweden, and the U.K. (all countries including the Channel Islands). Norway and Switzerland (not EU countries) also report findings of *P. ramorum.* Many findings have been in nurseries. Records outside of nurseries (including managed parks, gardens, public greens, woodlands, and forests) have arisen from Belgium, Denmark, France, Germany, Ireland, Luxembourg, the Netherlands, Norway, Slovenia, Spain, Switzerland, and the U.K. *P. ramorum* was also recently reported in Serbia (Bulajić and others 2009).

Asia–

It is speculated that *P. ramorum* may have originated from somewhere in Asia, such as the Yunnan, Taiwan, or the eastern Himalayas.

Distribution of Lineages (NA1, NA2, EU1) and Mating Types (A1, A2) Based Upon Isolate Testing

U.S. woodlands–

NA1, A2 and one EU1, A1 mating type isolate in a woodland stream in California

U.S. nurseries–

NA1, A2 and a few isolates of NA2, A2 and EU1, A1

Canada–

Not described, but some EU1, A1 isolates in British Columbia nurseries

Europe–

EU1, A1 and three EU1, A2 isolates in Belgium

Based upon genetic analysis, the NA1 and NA2 lineages are likely to have a separate geographic origin to the EU1 lineage and all three lineages are considered to have been introduced.

Risks from Entry of Exotic Lineages and Mating Types to the EU

Should NA1 and NA2 isolates enter the EU from North America, because they are of the A2 mating type, there is a risk of sexual reproduction with EU1 isolates. Any progeny that might be generated may show new adaptive behaviors and present new risks. There is uncertainty over whether the mating system is fully functional (data generated in the RAPRA project, WP4, and through other research work), but there is still potential for somatic recombination to occur. Until the origin/origins of *P. ramorum* is/are identified, there is another unquantifiable risk.

Current risks to the EU arise from: (1) the further establishment and spread of the EU1 lineage in EU MS, especially into the wider environment; (2) the introduction and spread of non-EU lineages from North America or from other unknown areas of origin; and (3) the introduction and spread of isolates of A2 mating type, regardless of lineage.

Major Pathways of Entry

We identified eight main **potential** pathways of entry for *P. ramorum* into the EU. Significant direct pathways are:

1. Plants for planting (PfP) (excluding seeds and fruit) of known susceptible hosts;
2. PfP (excluding seeds and fruit) of non-host plant species accompanied by contaminated, attached growing media;
3. Soil/growing medium (with organic matter) as a commodity; and
4. Soil as a contaminant (for example, on footwear, machinery).

Less significant direct/indirect pathways are:

5. Foliage or cut branches (for ornamental purposes) of susceptible foliar hosts;
6. Seeds and fruits of susceptible hosts;
7. Bark from susceptible hosts; and
8. Wood from susceptible hosts.

Probabilities of entry for each commodity type along with the associated level of uncertainty were assessed for the four geographical origins where *P. ramorum* has been recorded: U.S.; Canada; non-EU European countries (Norway and Switzerland, but not Serbia whose first finding post-dated the production of the PRA); and the unknown area or areas of origin for *P. ramorum*, speculated to be Asia – thus imports from China and Taiwan were included in the assessment. Although phytosanitary controls exist in both the emergency legislation for *P. ramorum* (Anonymous 2007) as well as the EC Plant Health Directive (Anonymous 2000), the assessment of the **overall** risk of entry was judged in the absence of these controls (this allows a reappraisal of the controls).

The EPPO scheme requires consideration of the probability of the ‘pest’ being associated with the commodity; the likely concentration of the ‘pest’ being high at the origin of the pathway accounting for cultivation practices, consignment treatments, and so on (excluding phytosanitary controls); and the volume and frequency of trade of the commodity on the pathway (trade data for 2003 to 2007 from the Eurostat Comext database). These data are very limited in their detail which led to a high level of uncertainty overall for pathway one – PfP of known susceptible hosts (see below), for which only rhododendrons and roses have specific data. Data

on PfP of non-hosts with associated growing media was generic, thus it was likely to include hosts of *P. ramorum*. Soil is prohibited entry into the EU (specifically soil and growing media containing soil or solid organic matter from certain countries – only Norway and Switzerland were considered potential sources of entry for *P. ramorum*) (pathway three); soil as a contaminant (pathway four) is obviously not documented. Data on foliage/cut branches of susceptible hosts (pathway five) was generic and so included non-hosts. Data for seeds and fruits of susceptible hosts (pathway six) were specified only for nuts of *Corylus* spp. and *Castanea* spp., and fruits of several *Vaccinium* spp. Data for susceptible bark (pathway seven) was not specified in the Eurostat database at all and so generic data for 'wood waste' was used. Data for known susceptible wood (pathway eight) were available for *Quercus* and *Fagus* spp. The probability of survival as well as multiplication of *P. ramorum* during transport and storage, and the probability of the pathogen remaining undetected during inspections were based upon data on the pathogen's biology generated in WP3 and 4 and in the literature. Distribution and end-use of the commodity in the EU, time of arrival, and likelihood of transfer of the pest to a suitable host/habitat, were also considered.

Host Plants and Plant Material on Which *P. ramorum* Can Move

For pathways one, five, six, seven, and eight, natural hosts were known to occur in 37 plant families, with 75 plant genera and more than 130 plant species affected (to 9 October 2008) (WP1). Results of experiments testing host susceptibility (WP2) predicted more potential hosts as well as some of the now known natural hosts. We did not consider potential hosts in our assessment of the risk of entry, since species which are only experimentally-susceptible cannot be regulated in the EU. Additionally, there are limited data generated in WP3 and from the literature on the susceptibility of fruits and the potential of fruits and seeds of various hosts to be significant pathways.

Table 1 summarizes the overall risk of entry by pathway using a five category rating.

Table 1—Estimated overall probability of entry for *P. ramorum* by pathway (PW) in the absence of phytosanitary controls. VL-Very Low; L-Low; M-Medium; H-High; VH-Very High

PW	Commodity	Pathway type	U.S.	Canada	Unknown origin	Europe (Non-EU)
1	Plants for planting (Hosts)	Direct	H	M	H	M
2	Plants for planting (Non-Hosts)	Direct	L	L	L	L
3	Soil as a commodity	Direct	M	M	M	M
4	Soil as a contaminant	Direct	L	VL	L	VL
5	Foliage/cut branches of susceptible hosts	Indirect	VL	VL	VL	VL
6	Seeds and fruits	Direct/ Indirect	VL	VL	VL	VL
7	Susceptible/ isolated bark	Direct	M	VL	M	VL
8	Susceptible wood	Indirect	L	VL	L	VL

In the absence of phytosanitary controls, the overall probability of further entry was considered to be high, mainly due to the wide host range and the ability of *P. ramorum* to persist in a variety of substrates (soil, growing media, bark, wood, foliage). Plants for planting of susceptible hosts (excluding seeds and fruits) from the U.S. and the unknown area/areas of origin represented the highest risk. The level of uncertainty for the overall probability of entry was low for all pathways with the exception of PfP for the unknown area/areas of origin, which was medium.

Assessment of the Risk of Further Establishment of *P. ramorum* in the EU

The assessment of the risk of establishment was based upon biological data arising from the RAPRA project, as well as a review of the literature and the deployment of various climatic matching techniques and models to produce a series of maps of potential establishment risk. *P. ramorum* has already been found on nurseries in many EU countries and although eradication has been feasible, *P. ramorum* has the potential to continue to become established in nurseries in the PRA area. Beyond nurseries, managed parks, gardens, woodlands, and now heathland (U.K.) have already become affected in parts of the EU. To determine the risk of further establishment, the EPPO scheme requires responses to a series of questions related to host range (known and experimental – WP1 and 2; in the WP1 database) as well as

the distribution of susceptible hosts and habitats in the PRA area. In this respect, the presence of sporulating hosts which are key to driving epidemics and which can lead to tree mortality through the development of stem cankers (for example, California bay laurel, *Umbellularia californica*, in California) had to be determined for the EU. In northern Europe, rhododendron has so far been the most important natural host in this respect, although RAPRA work (WP3) has identified the known natural hosts holm oak (*Quercus ile*x) and sweet chestnut (*Castanea sativa*) as possible inoculum sources for tree stem infection. *Vaccinium myrtillus* and other heathland species have been shown to be experimentally susceptible and potential sporulators for heathland and woodland habitats; *V. myrtillus* was recently identified as being naturally infected in the U.K. In southern Europe, epidemics in Mediterranean forests and in maquis shrubland have yet to be detected, but are likely to depend on evergreen foliar hosts such as *Q. ilex*, *Rhamnus alaternus*, and *Pistacia lentiscus*, shown in WP3 to support significant levels of sporulation. Questions on climatic suitability were tackled using observations of the abiotic requirements of the pathogen in the field as well as *in vitro* data from the RAPRA project (WP3) and the literature. Climatic comparisons of areas of the U.S. and the EU where *P. ramorum* is damaging plants (including trees), with the rest of Europe, was undertaken in RAPRA using CLIMEX (WP8). Because the area, or areas, of origin of *P. ramorum* are unknown, it is not possible to fully assess climatic favorability by this method. Comparisons between Oregon/California and Europe indicated that areas of northwest Spain, northern Portugal, southwest England, and parts of Italy and western Albania have the most similar climates. Larger parts of the U.K., Ireland, France, Belgium, the Netherlands, western Germany, Italy, the Adriatic coast of the Balkan peninsula, as well as north-west Turkey and east Bulgaria on the Black Sea coast, also have relatively good climate matches.

An additional approach to mapping establishment risk was undertaken using the methodology of Meentemeyer and others (2004) which has been used to predict potential *P. ramorum* distribution in California based upon a ranking system for climatic parameters which favor *P. ramorum* and a host species index. We were constrained in this work by the lack of high-resolution data for host distribution and host associations for the whole of Europe, and so could only deploy the climatic parameters. See fig. 2.

With respect to the semi-natural (including managed parks, gardens, public greens, and so on) or the natural environment, the parts of the PRA area that are most endangered based upon ranking of climatic factors alone are **Atlantic Central** and **Lusitanian** climatic zones. **Mediterranean** and **Atlantic North** climates are also potentially favorable, especially in coastal locations (see PRA for details). Although mild and wet climates are most likely to favor establishment and spread, the pathogen's ability to form long-lived chlamydospores enables it to survive Mediterranean climates with hot and dry summers, as demonstrated in California, and potentially also colder climates with cold winters. Areas of the EU with the most suitable climates coincide broadly with the areas that potentially have the most at-risk habitats, including potentially suitable broadleaved hosts/habitats, heathland and maquis areas. Those areas that are climatically favorable are only at risk where there are susceptible host plants that are capable of supporting sporulation, as tested in WP3. The most suitable predicted climatic locations for establishment based upon Meentemeyer and others (2004) are northern Portugal, northwestern Spain, the southern tip of Spain, the Adriatic coast of the Balkan peninsula (western parts of

Greece, Albania, Montenegro, Bosnia and Herzegovina, Croatia, Slovenia), southwestern France, northwest France (Brittany), northern coastal Spain, southern Turkey, western U.K., and southwest Ireland.

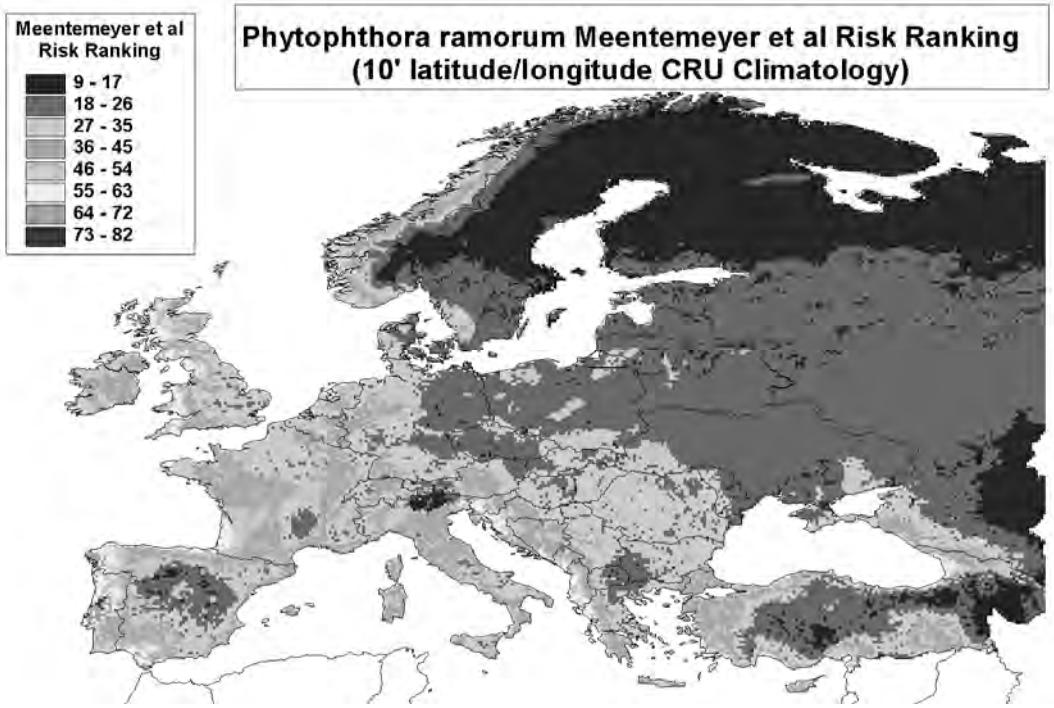

Figure 2—*P. ramorum* risk ranking model based on Meentemeyer and others (2004) for Europe (excluding host data), using 10' lat./long. resolution global climatology December to May 1961 to 1990.

With respect to susceptible hosts of cultivated shrubs and trees on nurseries, the whole of the PRA area is potentially endangered wherever these are produced because *P. ramorum* is favored by certain nursery practices (for example, overhead watering, use of contaminated irrigation water, use of chemicals containing metalaxyl-M for which some resistance has been identified, use of fungistatic compounds that may mask symptoms), and there are no sources of genetic resistance for an increasing list of hosts.

The main uncertainties arising in determining the risk of further establishment of *P. ramorum* in the EU are: mating system functionality – still uncertain (WP4); lack of high-resolution data on host distribution – this limited the determination of the endangered areas outside of nurseries (WP8); and rate of spread in the absence of phytosanitary controls. Other less significant uncertainties are listed in the PRA. The probability of further establishment in the PRA area is high.

Assessment of the Potential Economic (Including Environmental and Social) Consequences

The data that were used to complete this section of the PRA arose in part from a literature review, inquiries made to RAPRA partners and contacts in the U.S., plus a DEFRA impact assessment undertaken for the 2008 public consultation for the future management of *P. ramorum* (and *P. kernoviae*) in Great Britain. Additionally, within the RAPRA project (WP5), estimates of the current and future economic and environmental impacts of *P. ramorum* in three systems/scenarios were made. These were: the 'nursery system'; the 'northern European tree system' - broadly defined as trees with stem cankers in association with infected rhododendron in the Netherlands and the U.K.; and the 'southern European tree system' - a hypothetical system based upon the presence of the infected foliar host holm oak.

This section of the PRA proved to be particularly difficult, with varying degrees of uncertainty associated with the different elements that make up the overall assessment of potential impact. *P. ramorum* is subject to official control in the countries where it is known to occur; thus, the direct economic impact that it has caused is not quantifiable. Disentangling the costs of phytosanitary measures from the effect the pathogen has, or will have to be, estimated. In the EU, the intensity of *P. ramorum* surveys of nurseries, parks/managed gardens, woodlands, and so on has varied, and some countries have not reported survey findings to the EC, thus current impacts are underestimated. EU MS also vary in the availability of data on the costs of phytosanitary controls. Production and trade data for named host species grown in nurseries in the EU were not available, so generic data were used. Potential ecological and environmental impacts were estimated based upon the US experience. The impact in the area of origin/origins of *P. ramorum*, like the origin itself, is unknown. The potential for *P. ramorum* to establish in timber plantations in the EU is uncertain. For these reasons, financial estimates of the current and potential impact of *P. ramorum* were not possible for the EU. The scores assigned in responding to the questions in the EPPO scheme are subjective, and individual MS have/will vary in their assessment of the impact. However, the majority view of the potential impacts was presented in the PRA based upon the limited evidence that was available.

Current Impact of *P. ramorum* in the EU–

Currently *P. ramorum* has a direct effect on the quality of nursery stock as well as plants in managed parks and gardens. The current impact on nurseries in the EU is considered to be moderate in terms of quality and control costs (but excluding phytosanitary controls); including these controls, the impact is major. The current impact on plants in managed gardens is minor in many EU MS, but major in the southwest and west of the U.K., where damage in historic gardens is thought to be having a negative effect on tourism. In the natural/semi-natural environment of the EU, unlike the U.S., limited tree death has occurred only in the U.K. and the Netherlands since 2002. Heathland (*V. myrtillus*) has recently become affected in the U.K. In WP5, the current impact to the 'northern European tree system' is thought to be moderate as it is limited to a few parts of the EU and is fairly localized. In the 'southern European tree system' the current impact is minimal (zero) because *P. ramorum* is yet to be introduced there.

Potential (Future) Economic Impact in the EU–

Phytophthora ramorum has the potential to increase its host range and to become more widespread in the nursery trade and in the natural and semi-natural environment. The long-term potential for ecological damage is difficult to predict as the pathogen is considered to be at the start of the disease progress curve in the areas currently affected.

If phytosanitary controls are maintained at the current level or increased/reduced (but not removed), costs to nursery production and managed gardens will be major. Costs borne by National Plant Protection organizations will increase if increased controls are implemented to reduce further spread to the environment. However, there will be environmental benefits if controls focus on removal of foliar sporulating hosts that are invasive species, such as *Rhododendron ponticum* in the U.K., as planned for the new Food and Environment Research Agency (FERA) *Phytophthora* program.

Should phytosanitary controls be lifted globally, there will be an increase in production costs which will principally fall on nurseries producing hardy ornamental nursery stock (HONS) and on managed gardens. Quality effects on HONS will increase. These costs are major**.** Export losses may occur depending upon other countries' phytosanitary requirements.

In managed gardens (especially heritage plants in gardens involved in tourism), without control measures, effects on plant quality is likely to be moderate overall, but massive on a local scale. Social impacts may increase as a result of damage to plants in gardens visited by the public, potentially reducing visitor numbers, and ultimately affecting tourism where such gardens are part of that economy. Over all of the EU, the impact is likely to be moderate**.**

If controls are lifted, in the 'northern European tree system' the environmental impact will increase as the pathogen becomes more widespread in the environment, increasing the number of infected foliar hosts that sporulate, which may infect tree stem hosts - with potential for tree mortality. This impact has the potential to be major on a local basis, but moderate over the whole of the PRA area. In the 'southern European tree system', if *P. ramorum* is introduced, the impact would shift from minimal (zero) to major as the environment is considered to be highly favorable to the establishment of *P. ramorum*.

At-risk habitats that are yet to become affected by *P. ramorum* include most heathlands in northern Europe (the U.K. is now affected), as well as evergreen oak woodlands and laurel forests (laurisilva) and maquis/matorral habitats in southern Europe, but only where they contain susceptible hosts capable of sporulating, and favorable climatic conditions. Should these areas become affected, there will be knock-on effects on the ecology of the area.

The pathogen has yet to be found in timber plantations, but should it establish there long-term, the impact may be minor to moderate in the absence of controls.

Risk Communication

The final version of the PRA, dated 26 February 2009, was published online on the

RAPRA website (http://rapra.csl.gov.uk) and has been disseminated to the EC to help determine future phytosanitary requirements.

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Evidence of the Dynamic Response of Housing Values to a Sudden Oak Death Infestation[1]

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Abstract

Sudden oak death (SOD), caused by the non-indigenous forest pathogen *Phytophthora ramorum*, causes substantial mortality in coast live oak (*Quercus agrifolia*) and several other oak species on the Pacific Coast of the United States. Quasi-experimental hedonic models examine the effect of SOD on property values with a dataset that spans more than two decades including a decade of transactions before and after the invasion. The long study period allows for a unique contribution to the hedonic literature on natural hazards by studying the dynamic response of property values to an invasive species. The findings suggest property discounts of 2 to 5 percent for homes near infested oak woodlands, which are long lasting because of the continually dying oaks in the woodlands. Greater discounts of 5 to 8 percent occur if dying oaks are on the properties of homeowners, which are transitory because dying oaks are removed from homeowner properties. We compare recent hedonic modeling approaches including quasi-experimental, with spatial fixed-effects for a) communities, and b) parcels 'repeat sales', and spatial lag and error models to address bias from homeowner preferences, correlated with the price of a house and the proximity of a house to a SOD infection, which are not observed by the analyst.

Introduction

The recent arrival of several highly destructive forest pests and pathogens in the United States (for example, emerald ash borer, hemlock wooly adelgid, Asian longhorned beetle, and oak wilt) has increased public awareness of the dangers of forest invasive species. Reducing the damages from forest invasive species was the principal focus of the 2005 Public Land Corps Healthy Forests Restoration Act, which led to additional funding for the management of forest pests and pathogens. In particular, concern is focused on trees infected in residential areas because dying infected trees are an aesthetic and recreational dis-amenity, reduce ecosystem services (for example, screening, noise buffer, air quality, soil retention, and shade), and pose a physical hazard (for example, fire and falling trees) to nearby homes (Holmes and others 2009). There is limited information, however, of how homeowners respond to the damages over time from infestations of forest invasive species and what measures could be taken to mitigate those damages.

The goal of this study is to estimate the discounts over time to property values of an

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exotic forest pathogen (*Phytophthora ramorum*) in Marin County, California. This is accomplished with the quasi-experimental hedonic property value method. Sudden oak death (SOD) results in substantial mortality in several oak tree species on the Pacific Coast, and is believed to have entered the United States in the mid-1990s on nursery stock. The first noted mortality from SOD in the oak woodlands of Marin County was in late 1998. The data for the analysis cover more than 30,000 property transactions spanning more than two decades (1983-2008) across 56 communities within Marin County. The time span of the dataset encompasses the before (1983 to 1997) and after (1998 to 2008) period of the invasion when oaks in several study communities became infected with *P. ramorum*. This unique dataset permits a dynamic analysis of the dis-amenity effects on property values with results of the discount for each year of the invasion from 1998 to 2008. These results should prove useful in designing strategies for managing the damages of this invasion by informing extension specialists and arborists where to focus educational and removal of host plant efforts.

The hazards literature has assessed similar questions for natural disasters including other types of invasive species, wildfires, floods, and hurricanes. Horsch and Lewis (2009) used a quasi-experimental hedonic price function to examine the effect of an aquatic invasive species, and found a decrease in land values of 13 percent. Holmes and others (2006) and Huggett and others (2008) observed a discount of 1 percent and 8 percent, respectively, for properties with dying hemlock trees due to the forest invasive species, hemlock wooly adelgid. Donovan and others (2007) found that home prices are positively correlated with wildfire risk before information on wildfire risk is publicly available, whereas, afterwards, there is none. Chivers and Flores (2002) looked at the discounts associated with purchasing a home in a flood plain and found evidence of a discount only in years immediately after a flood event. Bin and Polasky (2004) observed a larger housing price discount for locating in a flood plain after Hurricane Floyd.

Study Area and Data

This study focuses on the property value effects of SOD on parcels within Marin County (fig. 1). As of 2008, the County had a population of 248,794. Marin County is located just north of San Francisco and is known for its natural beauty, liberal politics, and affluence. The interior is mountainous, forested, and largely undeveloped, while the eastern county along Highway 101 is suburban residential. Marin County has a per capita income of $51,950 and a median household income of $83,732, among the highest in the United States (United States Department of Commerce, Bureau of the Census. 2008).

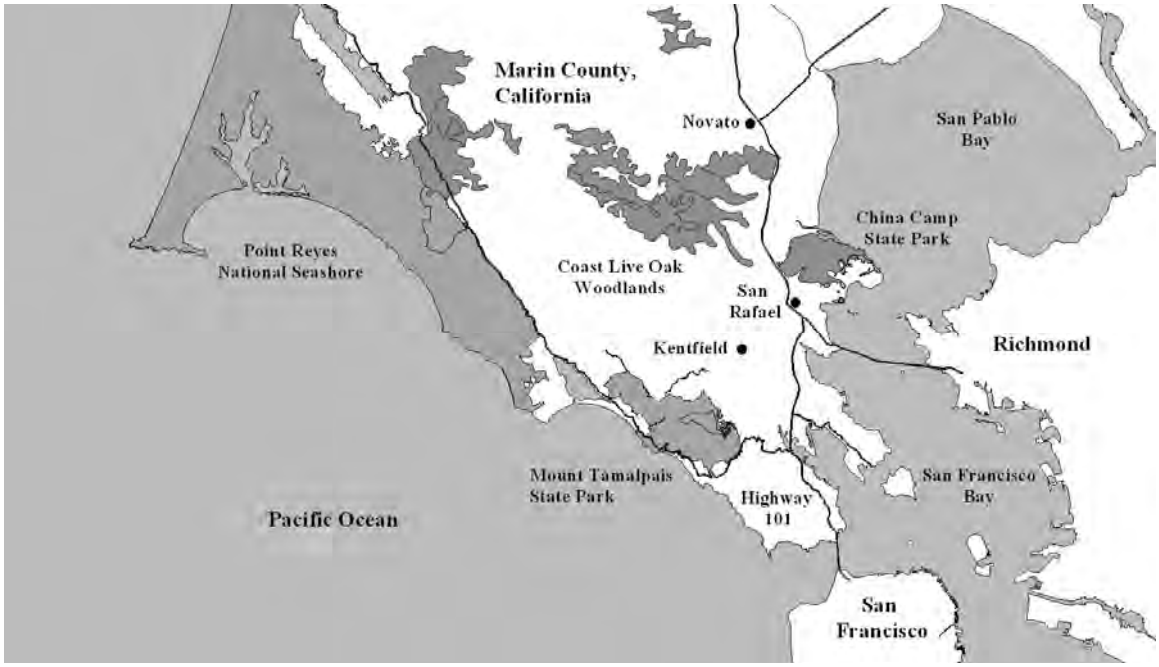

Figure 1—Map of study area.

Data and Variables for the Estimation

The data for this study are compiled from a variety of sources. Data on arms-length detached single-family home transactions are from the company CD-DATA, one of the largest providers of real estate information in California, which obtain data from county assessors. The data include the sale prices of the last three transactions for every property in Marin, in addition to lot and structure characteristics of every property. The hedonic application ultimately makes use of a subset of the property transactions for the years of 1983 to 2008. The entire panel of data represents transactions of 30,907 single-family homes in Marin County. The median sale price of the homes in inflation adjusted 2008 dollars is $807,467.

The literature does not provide complete guidance on the selection of variables or functional form in hedonic models, although in general, property prices are determined by the lot and structure and neighborhood characteristics. The dependent variable in all models is the observed arms-length transaction price adjusted to real dollars with the U.S. urban housing consumer price index (2008 dollars). Lot and structural characteristics include the age of the structure in years, the number of bedrooms (*BEDRMS*), the number of full bathrooms (*BATH*), the number of fireplaces (*FIREPL*), the acres of the lot area (*LOT*), the square footage of the building area (*BLDG*), indicator variables for the presence of a pool, more than one building, a garage, and central heating in the home, and an index for the quality of the structure of the home judged by the assessor (*QUAL*).

County GIS spatial data are from MarinMap, a consortium of public agencies (local governments, special districts) organized under the Marin General Services Authority.[5] To alleviate omitted variable bias, a variety of neighborhood variables are

[5] For more information, see http://marinmap.org.

calculated from this GIS data. We identify 56 distinct communities[6] in Marin County defined by the Community Development Agency of the County of Marin.[7] The hedonic models during the period of the SOD invasion include 56 indicator variables for each community, and the panel identifier for the community fixed-effects difference-in-differences model is the 56 communities.

Neighborhood variables for location include the number of feet from the Golden Gate Bridge (which links Marin County with San Francisco), the closest town center, including interaction variables with indicator variables for 10 large towns in Marin County,[8] and the second closest town center, including interaction variables with indicator variables for the same 10 towns.

Additional neighborhood variables include indicator variables for i) quarter-mile proximity to major roadways, bus routes, noise contours, libraries (*DLIB)*, highways (*DHWY*), historic sites (includes the closest and second closest); ii) half-mile proximity to an airport, ferry hubs (*DFERY)*, county facilities, district offices, park 'n rides, fire stations, schools, medical facilities, non-economical mineral deposits; and iii) within a dam inundation zone, a floodplain, school districts (includes four variables for the districts, with the San Rafael District omitted), landslides zones (includes four variables of landslide frequency, with water area omitted), earthquake zones (includes five liquefaction[9] potential zones, with wave liquefaction omitted).

Relevant natural amenity variables include the number of inches of precipitation (*PRECIP)*, the elevation of the property about sea-level (*ELEV)*, indicator variables for i) quarter-mile proximity to the ocean, streams, rivers, lagoons, lakes, neighborhood parks, ridge way greenbelt, federal parks, redwood woodlands; ii) half-mile proximity to wetlands (estuarine, palustrine 'emergent, forest, unconsolidated, farmed', with marine omitted), neighborhood parks, ridge way greenbelt, federal parks; and iii) mile proximity to neighborhood parks (*DPRK*), ridge way greenbelt, federal parks, McInnis County Park (*DMCINN)*, China Camp State Park (*DCHINA*), and the Golden Gate National Recreation Area (*DGNRA*).

Sub-regions of Marin County may experience different housing market conditions over time. For instance, the southern County may have a faster price increase, while the northern County, a slower price increase. To alleviate bias of time-varying omitted variables, we control for potentially "hot/cold" regional housing markets. An

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[6] These communities are Belvedere, Larkspur, Mill Valley, Novato, San Rafael, Sausalito, Corte Madera, Fairfax, Ross, Tiburon, San Anselmo, Dillon Beach, Tomales, Northern tip of Eastshore, Eastshore, Forest Knolls, Olema, Pt. Reyes Station, Inverness, San Geronimo Village, Muir Beach, Woodacre, Muir Woods Park, Alto, Lucas Valley, Country Club, Point San Pedro, Los Ranchitos, Homestead, Waldo Point, Paradise Cay, Unincorporated Fairfax, Santa Venetia, Greenbrae Boardwalk, Bayside Acres, California Park, San Quentin, unincorporated Tiburon, Marin City, Almonte, Tamalpais, Strawberry, Sleepy Hollow, Bel Marin Keys, Loma Verde, St. Vincent's, Kentfield, Stinson Beach, Lagunitas, San Geronimo Valley, Sun Valley, Black Point, Bolinas, Nicasio, Indian Valley, North Novato, South Novato, Lucas Valley Environs, Marinwood.

[7] The Current Planning Division of the Community Development Agency of the County of Marin administers and enforces zoning and subdivision regulations in accordance with the Marin Countywide Plan and applicable state laws.
http://www.co.marin.ca.us/depts/CD/main/comdev/CURRENT/index.cfm

[8] Belvedere, Larkspur, Mill Valley, Novato, San Rafael, Sausalito, Corte Madera, Fairfax, Ross, and Tiburon (San Anselmo omitted) each have populations greater than 2,000.

[9] Liquefaction describes the behavior of soils that suddenly transition from a solid state to a liquefied state, such as during earthquakes.

indicator variable for the northern communities (*DNORTH*) is interacted with indicator variables for the years 1996 to 2008 (*DNORTH00, DNORTH05*). Also, an indicator variable for the southern communities (*DSOUTH*) is interacted with indicator variables for the years 1996 to 2008 (*DSOUTH00, DSOUTH05*).[10]

A unique feature of the dataset is the presence of the last three transaction prices for every property in the County. Most properties sold more than once during the study period from 1983 to 2008. Since properties selling more than once potentially have different homeowner characteristics than properties selling only once, we include indicator variables for properties that sold twice (11,204 transactions) or three (12,486 transactions) times during the study period. Since the study period includes the 2000 to 2006 housing boom in the United States, when there was significant speculative behavior, the indicator variables for the properties that sold twice or three times are interacted with time dummies for 1996 to 2008.

Sudden Oak Death Variables for the Estimation

We account, with SOD indicators, for the presence/abundance of SOD infections with indicator variables for quarter-mile proximity to i) coast live oak woodlands (*OAKWOOD*), ii) confirmations of SOD infections of coast live oak (*CONFIRM*), iii) oak dieback from a 2005 aerial survey by the U.S. Department of Agriculture, Forest Service (USDA FS) (*AERIAL*),[11] and iv) arborist's records of neighborhoods in Novato, San Rafael, and Kentfield with heavy damage from SOD (*ARBOR-NV, ARBOR-SF, ARBOR-KF*). Generic reference to any one of the SOD indicators is *SODID*.

Mortality in the woodlands (*OAKWOOD*) is a concern to homeowners close to the woodlands because of reduced aesthetic, ecosystem service, and recreation values, in addition to posing a physical hazard. *CONFIRM* and *AERIAL* are a concern to homeowners because dying oak trees are on a homeowner's property or an adjacent neighbor's property. Homeowners in heavily damaged neighborhoods (*ARBOR-NV, ARBOR-SF, ARBOR-KF*) cope not only with dying trees on their own property, but also on adjacent neighbor's properties and in nearby oak woodlands.

County GIS data for the location of coast live oak woodlands and confirmations of SOD infections are from the University of California, Berkeley's Geospatial Innovation Facility.[12] County GIS data for the 2005 aerial survey are from the USDA FS Pacific Southwest Region.[13] The location of neighborhoods where there was heavy oak mortality is from a 2008 telephone survey of arborists in Marin County.[14]

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[10] The northern communities include Novato, Bel Marin Keys, Black Point, Indian Valley, Loma Verde, North Novato, and South Novato. The southern communities include Belvedere, Mill Valley, Sausalito, Tiburon, Almonte, Alto, Homestead, Marin City, Muir Woods, Paradise Cay, Strawberry, Tamalpais, and Unincorporated Tiburon.

[11] California GIS maps of SOD confirmations and aerial surveys of oak dieback are publicly available on the OakMapper. For more information, see http://oakmapper.org/.

[12] For more information, see http://giifserv.cnr.berkeley.edu/website/OakMapper/metadata/species.htm and California Gap Analysis, and http://giifserv.cnr.berkeley.edu/website/OakMapper/metadata/sod.htm and the Kelly research and outreach lab.

[13] For more information, see http://www.fs.fed.us/r5/spf/fhp/fhm/sod/index.shtml. We thank Zachary Heath for supplying this data.

[14] The information about the neighborhoods with heavy oak mortality came mostly from Bartlett Tree Service in Marin County. For more information, see http://www.bartlett.com/index.cfm. The tree services polled their arborist for the top spots in each of their geographically based areas of work.

The arborist's records do not indicate the years when the oak mortality occurred. However, the 2001 to 2002 and 2005 to 2007 aerial surveys from the USDA FS of oak dieback allow for an approximation of when the mortality in the neighborhoods occurred.

Coast live oak woodlands shown in fig. 1 are principally in the central and eastern regions of the County near San Rafael, but there are also smaller woodlands in the northern region near Novato. Mortality in the woodlands began in Marin in late 1998 and continues to this day. As of 2008, there were 33 SOD confirmations throughout the neighborhoods of Marin, and nearly all of the confirmed samples were taken in 2000 and 2001. Aerial surveys of oak dieback by the USDA FS are available for the years 2001 to 2002 and 2005 to 2007, but the oak dieback from the years other than 2005 is either too coarse (2001, 2002) or too far away from most of the property transactions (2006, 2007) to be useful in this study. There are 29 distinct patches of oak dieback in 2005, with the largest patches of dieback in the central and southern regions near San Anselmo, Fairfax, San Rafael, Tiburon, and Sausalito, but also smaller patches in the northern region near Novato.

The neighborhoods with heavy damage from SOD are based on sections of streets in the towns of Novato, San Rafael, and Kentfield where arborists identify significant tree removals due to SOD.[15] The neighborhoods in Novato and San Rafael with heavy damages due to SOD are also beside large tracts of infested oak woodlands, while the neighborhoods in Kentfield are not. We suspect heavy damages in the neighborhoods of Novato, San Rafael, and Kentfield starting in 2002, 2000, and 2005, respectively, based on the USDA FS aerial surveys that indicate oak dieback near those neighborhoods starting in those years.

The vector of variables *OWIMPACT* is the interaction of *OAKWOOD* and year-specific dummies from 1996 to 2008. The coefficient estimates for the vector of variables *OWIMPACT* indicate the premium/discount to property values of proximity to *OAKWOOD* from 1996 to 2008. Two years prior to the 1998 invasion are included to examine what property value premium/discount exists before the invasion. The vector of variables *CFIMPACT* is the interaction of *CONFIRM* and dummies for two-year intervals from 1996 to 2008. The dummies are in 2-year intervals because of the limited number of property transactions in *CONFIRM* in each year, preventing accurate statistical estimation for *CONFIRM* interacted with year-specific dummies.

The vector of variables *AEIMPACT* is the interaction of *AERIAL* and year-specific dummies for 2004 to 2008. There are year-specific dummies for only 5 years because there is no expectation of visible SOD infections in *AERIAL* prior to the 2005 aerial survey. The vector of variables *ARIMPACT-NV*, *ARIMPACT-SF*, and *ARIMPACT-KF* is the interaction of *ARBOR-NV*, *ARBOR-SF*, *ARIMPACT-KF*, and year-specific dummies for 2000 to 2008, 1998 to 2008, and 2003 to 2008, respectively, based on aerial surveys that indicate oak dieback in those time frames.

[15] The Novato locations include Indian Valley Rd., Wild Horse Valley Rd., Ignacio Blvd., Pacheco Creek Rd., Oak Forrest Rd., and the Alameda del Prado. The San Rafael locations include Convent Ct., Oakdale Dr., North San Pedro Rd., and Bret Harte Rd. The Kentfield locations include Woodland Rd., Upland Rd., and Crown Rd.

Methods

A number of functional forms are considered for the hedonic models. All specifications have a very similar fit, with the linear Box-Cox (constant lambda transformation on non-binary independent variables) fitting just slightly better than a semi-logarithmic model. We chose the semi-logarithmic model because of its prevalence in the literature and ease of interpretation. Pair-wise correlation analysis and calculation of variance inflation factors fail to indicate that multicollinearity is a serious problem. Lastly, White's robust standard errors are used for all models, to account for potential heteroskedasticity.

The difference-in-differences model uses the study period 1983 to 2008. Our quasi-experimental strategy exploits the substantial spatial and temporal variation present in this longer study period that includes transactions before and after the SOD invasion.

The full dataset for the study period 1983 to 2008 consists of a total of 30,907 observations, spanning 56 communities. The price of parcel *i* on community *j* in time *t* take forms:

Ordinary Least Squares (OLS):[16]

$$\ln P_{it} = X_i'\beta + Z_{it}'\phi + \delta_1 SODID_i + IMPACT_{it}'\delta_2 + T_t'\delta_3 + \varepsilon_{it} \quad (2)$$

Community Fixed-Effects:

$$\ln P_{it} = X_i'\beta + Z_{it}'\phi + \delta_1 SODID_i + IMPACT_{it}'\delta_2 + T_t'\delta_3 + \alpha_{j(i)} + \varepsilon_{it} \quad (3)$$

A subset of the full dataset consists of 23,690 transactions of only the properties that sold more than once during the period of 1983 to 2008.

Parcel Fixed-Effects 'Repeat Sales':

$$\ln P_{it} = Z_{it}'\phi + IMPACT_{it}'\delta_2 + T_t'\delta_3 + \alpha_{i(t)} + \varepsilon_{it} \quad (4)$$

where X_i is a Kx1 vector of time-constant variables specific to parcel *i*, Z_{it} is a Lx1 vector of time-varying variables specific to parcel *i*, T_t is a Jx1 vector of year-specific dummy variables, and *SODID* and *IMPACT* identify the difference-in-differences effect of SOD (discussed below). In (3), $\alpha_{j(i)}$ is a community specific fixed-effect, potentially correlated with the regressors, associated with community *j* where parcel *i* is located. In (4), $\alpha_{i(t)}$ is a parcel specific fixed-effect, potentially correlated with the regressors, associated with parcel *i* occurring at time *t*.

The spatial difference-in-differences specification estimates the effects of SOD on property values from the year the invasion starts, which varies depending on SOD indicator, to the end of the study period in 2008. The coefficient for $SODID_i$ (δ_1) is the premium/discount of properties in places eventually affected by SOD, before the invasion begins. The coefficients on $IMPACT_{it}$ (δ_2)[17] specify the discount to the values of properties affected by SOD just before and after the invasion is underway.

[16] Estimation of a community random-effects model yields results identical to ordinary least squares.

[17] $IMPACT_{it}$ is a vector of interaction variables of the $SODID_i$ indicator and year-specific dummies for the years just before and after the SOD invasion begin. For *OAKWOOD CONFIRM*, *AERIAL*, *ARBOR-NV*, *ARBOR-SF*, and *ARBOR-KF*, the year-specific dummies are for the years 1996 to 2008, 1996 to 2008 (in 2-year intervals), 2004 to 2008, 2000 to 2008, 1998 to 2008, and 2003 to 2008, respectively.

The coefficients estimates (δ_2) are the difference-in-differences components of interest.[18]

Fixed-effects are not present in the error term, and so consistent parameter estimates are possible even if correlation exists between the fixed-effects and the independent variables. The definition of the spatial fixed-effect is typically political and demographic boundaries similar to those of census tracts (for example, Pope 2008a, 2008b).[19] In our application, the most plausible argument for the spatial relationship between properties is that of the community defined by the Community Development Agency of Marin County. One would expect error terms to be correlated within a community because many community-specific characteristics are shared.

Parcel fixed-effects are a true panel approach, often referred to as 'repeat sales,' that uses the same houses that have sold multiple times over the study period. Most of this unique dataset consists of properties that sold twice (11,204 transactions) or three times (12,486 transactions) during the study period. The ability to observe the transaction price of the same house in differing time periods increases the flexibility of the researcher for controlling for unobserved spatial heterogeneity.[20] The parcel fixed-effects specification has fewer variables than the community fixed-effects model because any time-constant parcel variable is absorbed by the fixed-effect. Only variables that vary over time for the parcel are estimated.

The last econometric issue to discuss is the use of a 25-year time-series of property sales. To account for basic temporal dependency, we include a vector of dummy variables T_t to specify the year a given transaction takes place. To control for price-differentials over time across sub-regions, we include interaction terms of the indicator variables *DNorth* and *DSouth* and year-specific dummies for 1996 to 2008. To account for time-varying speculative behavior during the housing boom, we include interaction terms of indicator variables for properties that sold twice or three times and year dummies for 1996 to 2008.

Results

Table 1 summarizes the results from the spatial difference-in-differences model, where the community (3) and the parcel (4) fixed-effects forms, for control of community and parcel specific effects, are shown after ordinary least squares (2). The results are very similar across the estimations with the ordinary least squares having a slightly better fit because all time-constant variables are included. The stability of coefficients across the estimations indicates a degree of model robustness.

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[18] To see this, suppose two time periods and two infestation levels. $P_{T,YI}$ is the price of a property in proximity to an eventual SOD infestation (T for treatment) and in a year of the infestation (YI), and $P_{C,YN}$ is the price of a property not in proximity to an eventual SOD infestation (C for control) and in a year prior to the infestation (YN). The difference-in-differences component of interest is: $(P_{T,YI} - P_{T,YN}) - (P_{C,YI} - P_{C,YN}) = ((\delta_1 + \delta_2 + \delta_{3,YI}) - (\delta_1 + \delta_{3,YN})) - ((\delta_{3,YI}) - (\delta_{3,YN})) = \delta_2$.

[19] A challenge lies in determining the appropriate geographic resolution for the spatial fixed-effects. If the geographic resolution is too coarse, the fixed-effects may fail to absorb meaningful variation in the omitted variables. If they are too small, they may absorb most of the variation in the characteristic of interest (Kuminoff and others 2009).

[20] Palmquist (1982) has a general discussion of using repeat sales data to estimate environmental characteristics.

The coefficients of the non-SOD variables are generally stable across the estimations. For instance, the coefficients on *LOT*, *BLDG*, *QUAL*, *DHWY*, and all the *TIME* variables are nearly identical and of the same order of statistical significance. *PRECIP* and *DCHINA* are controls of interest because rainfall is a pathway of spread for SOD and China Camp State Park was an early epicenter for SOD infections in Marin County. The coefficients for these variables are robust, statistically significant, and have their expected sign across the models.

The results for *OAKWOOD* and *OWIMPACT* have the expected sign and are significant, generally counter to the results for the models only using transactions for the period of the SOD invasion. Prior to the invasion, the *OAKWOOD* coefficient indicates proximity to oak woodlands has no statistically significant effect on property values.[21] After the invasion, the results for the 2000, 2004, and 2008 *OWIMPACT* coefficients indicate statistically significant discounts of 3 to 5 percent.

The results of *OWIMPACT* are generally stable across estimations (two, three, and four) in sign and magnitude. The indicator variables for properties that sell multiple times during the study period sell indicate these properties sell at a premium over properties with only one sale. This indicates the 'repeat-sales' model may represent a different type of housing market, with more speculative behavior, that is more susceptible to time-varying trends from the U.S. housing boom. The community fixed-effects model appears to resolve issues of bias and inefficiency from spatial unobservables, while providing some resistance to trends from the boom since homes that sold only once are included. Because of these advantages, only the results of the community fixed-effects model are displayed for the other SOD indicators.

[21] The community fixed-effects estimation indicates a slight premium may exist for homes beside healthy oak woodlands, which matches expectations.

	OLS		Community Fixed-Effects		Parcel Fixed-Effects "Repeat Sales"	
	Coef.	**Robust t-stat**	**Coef.**	**Robust t-stat**	**Coef.**	**Robust t-stat**
Constant	12.35**	146.66	11.88**	94.26	13.18**	1092.39
LOT	4.47e-7**	7.28	4.03e-7**	6.50	--	--
BLDG	1.86e-4**	36.67	1.82e-4**	36.47	--	--
BATH	0.01	1.35	0.01	0.81	--	--
BEDRMS	0.02**	6.89	0.02**	7.29	--	--
FIREPL	0.01	0.79	0.01	1.42	--	--
QUAL	0.12**	34.05	0.11**	31.43	--	--
DHWY	-0.03**	5.89	-0.02**	3.81	--	--
DFERY	0.01	0.43	0.02	0.92	--	--
DLIB	0.03**	3.18	0.02**	2.82	--	--
PRECIP	-4.63e-3**	11.47	-2.18e-3**	3.57	--	--
ELEV	-2.02e-5	1.27	-2.01e-5	1.21	--	--
DPRK					--	--
Mile	-0.03	0.46	0.11	0.98		
DMCINN	0.01	0.40	0.08**	2.92	--	--
DCHINA	0.09**	4.22	0.09**	3.39	--	--
DGNRA	-0.02	0.91	-0.01	0.11	--	--
TIME						
1992	0.08**	5.14	0.08**	5.24	0.09**	5.61
1997	0.02	1.18	0.02	1.21	0.03*	1.29
2002	0.52**	35.20	0.51**	36.23	0.51**	29.76
2007	0.73**	49.96	0.73**	51.19	0.84**	41.60
DNORTH	0.04	1.62	--	--	--	--
2000	-0.09**	6.61	-0.10**	7.07	-0.10**	6.43
2005	-0.04**	3.42	-0.04**	3.24	-0.08**	4.88
DSOUTH	-0.02	1.79	--	--	--	--
2000	0.10**	5.55	0.10**	5.40	0.12**	5.82
2005	0.02	1.01	0.01	0.89	0.01	0.59
OAKWOOD	-0.01	0.08	0.02	1.21	--	--
OWIMPACT						
2000	-0.05*	2.68	-0.05*	2.58	-0.04*	1.98
2004	-0.03	1.95	-0.03*	2.15	-0.03	1.32
2008	-0.05*	2.04	-0.05	1.86	-0.08**	2.77
N	30,907		30,907		23,690	
R^2	0.75		0.68		0.70	
Panel ID	--		56 Communities		9,764 Parcels	
Rho	--		0.336		0.776	

Table 1—Estimation results for spatial difference-in-differences hedonic models (1983 to 2008) – SOD indicator (*OAKWOOD*)

Note: * ** indicate significance at the 95 percent and 99 percent levels. Models use the semi-log functional form. Median home sale price in real 2008 dollars is $807,467.

Table 2 displays the results for difference-in-difference community fixed-effects model to examine the percentage discount to property values over time, for each of the SOD indicators. Given that the study period spans 25 years, estimation is possible of the dynamic path of the discount to property values of proximity to a SOD indicator by year for more than a decade. The shaded cell of each column of Table 5 indicates the year the SOD indicator is expected to start detecting discounts to property values from the invasion. Two years of results prior to the year of expected detection are shown to compare results before and after the invasion.

Table 2—Dynamic response of property values to the SOD invasion (by percent change) for each SOD indicator

Year	Coast oak woodland (OW IMPACT)	SOD confir-mations (CF IMPACT)	2005 Aerial Survey Mortality (AE IMPACT)	Novato Neigh-borhoods (ARIMPACT-NV)	San Rafael Neigh-borhoods (ARIMPACT-SF)	Kentfield Neigh-borhoods (ARIMPACT-KF)
1996	4.50 (1.53)	-8.15 (0.80)	--	--	--	--
1997	-0.10 (0.04)		--	--	--	--
1998	-0.01 (0.03)	-3.92 (0.44)	--	--	-4.21 (1.19)	--
1999	-3.15 (1.22)		--	--	-6.67 (1.55)	--
2000	-4.30* (2.39)	-11.04 (1.49)	--	1.21 (0.36)	-8.52** (3.33)	--
2001	-1.69 (0.91)		--	4.92 (1.49)	-8.79** (2.72)	--
2002	-1.49 (0.97)	-1.78 (0.49)	--	-1.69 (0.57)	-7.69** (2.93)	--
2003	-4.21** (2.61)		--	-6.57 (1.19)	-6.85* (2.35)	1.92 (0.33)
2004	-3.15* (2.13)	-1.49 (0.52)	2.12 (0.77)	-2.27 (0.97)	-8.24** (3.18)	-4.30 (0.74)
2005	-1.78 (1.18)		-1.29 (0.48)	-8.70** (4.17)	-6.20* (2.48)	-15.46 (1.96)
2006	-3.34* (2.27)		-6.67* (2.01)	-6.76* (2.48)	-6.95** (3.03)	-17.88** (2.89)
2007	-0.80 (0.54)	5.55 (1.27)	-0.20 (0.11)	1.71 (0.58)	-10.06** (4.61)	-1.59 (0.24)
2008	-4.02 (1.68)		2.02 (0.36)	5.55 (1.27)	-15.80** (3.57)	10.74 (1.13)
R^2	0.68					
Panel ID	56 Communities					
Rho	0.33					

Number of observations: 30,907. Note: * ** indicates significance at the 95 percent and 99 percent levels. These are the community fixed-effects difference-in-difference hedonic models. Robust t-statistics are in parentheses. Shaded cells indicate the year when visibly dying trees are expected to first appear for each of the indicators.

OWIMPACT results indicate a discount of 3 to 5 percent for every year from 1998 to 2008, with these discounts significant in the years 2000, 2003, 2004, and 2006. Note that in 1996, prior to the invasion, there is a premium of 4 to 5 percent, which suggests an even deeper discount may have occurred. The continually dying oaks in the woodlands have an ongoing effect on the discount to property values. This likely persists until there are no further dying oaks in the vicinity of the homes. These discounts are less severe than if the dying oaks are located on the homeowner's property.

CFIMPACT results indicates a large, 11 percent, though statistically insignificant, discount in the years of 2000 and 2001.[22] The lack of statistical significance is likely because of the small number of transactions in quarter mile proximity to the locations confirmed to have SOD. The magnitude of the discount fades in subsequent years. Note that the presence of a discount prior to invasion for *CFIMPACT* means the discount may not be as high as 11 percent. The 2006 coefficient for *AEIMPACT* indicates a discount of 6 to 7 percent, statistically significant, on homes near oak dieback observed in the 2005 aerial survey. This discount fades and eventually switches to a statistically insignificant premium in 2008. This suggests property values rebound after the dying oaks are removed from a homeowner's property.

ARIMPACT-NV, SF, and *KF* results indicate that heavily damaged neighborhoods produce large and often ongoing discounts on nearby property values.[23] For the Novato neighborhoods, the discounts, generally between 6 to 8 percent, last for 5 years following the invasion, and are statistically significant for 2 of the years. The discounts for the San Rafael neighborhood, generally between 6 to 15 percent, last to the end of the study period, and are all statistically significant. Note that discounts are present in the San Rafael neighborhood prior to the invasion, which suggests the discounts from SOD may not be as high as 15 percent. However, two large discounts in the Kentfield neighborhoods, close to 15 percent, also coincide with the invasion.

The difference in the number of years of discounts in the neighborhoods is related to their proximity to infested oak woodlands and how long the woodlands were infested. The San Rafael neighborhood is in proximity to China Camp State Park, where SOD mortality has been severe since 1998. Homeowners have observed dying oaks on the hillsides of the park for a decade. The Novato neighborhood is also in close proximity to oak woodlands, although the infestation began later and was less severe than in China Camp State Park. The Kentfield neighborhoods are not in close proximity to open areas of oak woodlands.

Discussion

The findings of this study indicate the dynamic effects on property values in Marin County, California from an invasion by the forest invasive species, *P. ramorum*. We use a quasi-experimental hedonic model for the study period 1983 to 2008, with the first large wave of SOD mortality in Marin County in late 1998, to detect the discounts from proximity to dying trees in oak woodlands, properties of homeowners, and heavily damaged neighborhoods. Properties within a quarter-mile from SOD infested oak woodlands experience a 2 to 5 percent discount, and this discount is ongoing since oaks are continually dying in the woodlands. If dying oaks are on a homeowner's property, we observe a greater discount of 5 to 8 percent, though this discount is transitory and significantly diminishes or completely disappears within a couple years. The most severe discounts of 8 to 15 percent occur, which can last for several years or longer, if dying oaks are throughout a neighborhood and in nearby woodlands.

[22] For 230 of the samples collected at locations in Marin County and later confirmed to be positive for SOD, 204 of those samples were collected in 2000 and 2001.
[23] The years of discounts closely correspond to the dates when oak dieback is observed in the USDA FS aerial surveys.

Government agencies' spending on invasive species management is significant, despite the general lack of estimates of the damages over time of the invasions, from a rigorous economic framework (Olson 2006). Our results indicate that government spending on homeowner education of the symptoms of SOD and on the removal of dying trees is crucial for mitigating property value discounts. Education about SOD helps homeowners to realize when an infestation is present and contact government or private arborists about removing infected plants before the disease grows worse and spreads to other oaks. Property value discounts are most severe and long lasting for heavily damaged neighborhoods near infested oak woodlands, where dead and dying oaks are left standing, and these discounts could have been avoided or mitigated by faster removal of the dead and dying oaks.[24]

Many natural hazards (for example, wildfires, floods, hurricanes, and invasive species) have long-lasting effects on property values, and more studies examining the dynamic response of property values to natural hazards are needed. Understanding how natural hazards cause damages over time is important for improving the government response with education and management. More generally, the dynamic response to changes in resources suggests how the people value resources over time and, thus, broadly informs long-run policies involving them.

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[24] Because infected oaks do not spread the pathogen and there is a high cost associated with removal of oaks, many dead and dying oaks are left standing in open oak woodlands.

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Biology II

Ancestral Seed Zones and Genetic Mixture of Tanoak[1]

Richard Dodd,[2] Zara Rafii,[2] and Wasima Mayer[2]

Abstract

Understanding the genetic structure of tanoak (*Lithocarpus densiflorus*) is necessary to pathologists seeking natural variation in resistance to *Phytophthora ramorum*, cause of sudden oak death (SOD), and to resource managers who need indications of conservation priorities for this species now threatened by this introduced pathogen. We investigated population genetic structure using nuclear and chloroplast DNA in 43 populations from throughout the range of the species. Our chloroplast DNA results revealed four major and two rare haplotypes. The 4 major haplotypes delineated ancestral seed pools from central and northern coastal California, extreme northern California and Oregon, Klamath Mountains and the Sierra Nevada. Diversity at nuclear microsatellite loci supported the chloroplast lineages and indicated some further divergence within them. We propose that at least six breeding zones should be recognized for disease resistance screening and for conservation management. These include 1. southern-most populations from Nacimiento, Lompoc, and Santa Barbara that are relatively low in genetic diversity; 2. central coastal populations from Big Sur to the San Francisco peninsula; 3. populations from north of the San Francisco Bay to Arcata; 4. extreme northern California and Oregon populations from north of Arcata to the northern limit of the species' range; 5. Klamath Mountains; and 6. the Sierra Nevada.

Introduction

The sudden oak death (SOD) epidemic, caused by *Phytophthora ramorum*, has caused considerable damage to natural woodlands of central and northern California. Keystone species such as tanoak (*Lithocarpus densiflorus* [Hook. & Arn.] Rehder, Fagaceae), recently attributed to a new genus (*Notholithocarpus densiflorus* [Hook. & Arn.] Manos, Cannon & S. Oh), and coast live oak (*Quercus agrifolia* Née) have suffered the highest mortality. Because of the dominance of these species in the coastal mixed evergreen forests, their loss will have significant impacts on the composition, function and management of these coastal ecosystems. Although the disease is believed to be absent from north-western California, the extensive woodlands of tanoak there and in southern Oregon are likely to be highly sensitive to the disease because of suitable climatic conditions for the pathogen and the dominance of tanoak.

Studies of host resistance to SOD indicate quantitative variation in susceptibility (Dodd and others 2005, 2008; Hayden and others, unpublished data), so it seems likely that variations in host tolerance will play a crucial role in the future of the coastal ecosystems. The observed variation in susceptibility indicates that genetic variation is present in natural populations and so resistance screening is a worthwhile

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endeavour. Initial work on population genetic variation in tanoak (Nettel and others in press) has guided the choice of populations in an effort, currently underway, to detect resistance within a limited number of populations. An understanding of genetic structure of susceptible host species is crucial in implementing resistance screening. Where populations are at risk of extinction, as is the case for tanoak, discovery of divergent population lineages will also provide a rationale for conservation priorities.

Here we report on a detailed analysis of genetic variation in nuclear and chloroplast DNA covering almost the entire range of tanoak. Our choice of genetic markers was predicated on the need to obtain the maximum amount of information on levels of genetic diversity and to identify seed lineages that might be important in demarcating potential seed zones. In tanoak, chloroplast DNA is inherited clonally through a maternal lineage. Genetic variation in chloroplast DNA is typically low, but it provides an excellent system for detecting the limits of seed dispersal. On the other hand, nuclear microsatellites are highly polymorphic and provide an excellent means of assessing variations in levels of genetic diversity.

Materials and Methods

Sampling and DNA Analysis

Leaves from mature individuals of *L. densiflorus* ssp. *densiflorus* and one population of dwarf tanoak *L. densiflorus* ssp. *echinoides* were obtained from coastal populations from Coos County, Oregon to Santa Barbara, California, and from the southern Klamath Mountains and the Sierra Nevada (table 1). We sampled a total of 905 trees from 43 populations (fig. 1), with an average of 21 trees per population. Leaves were maintained frozen at –20 ºC. We extracted DNA using a simplified CTAB method (Cullings 1992).

Chloroplast DNA

After testing more than 40 putative loci, we detected five polymorphic cpDNA mononucleotide microsatellites in tanoak (table 2): *ccmp4* (*atpF* intron, Weising and Gardner, 1991), *µcd2_p14* (*trnC-ycf6*, Deguilloux and others 2003), *Cmcs5* (*ndhG-ndhI*, Sebastiani and others 2004), *KI* (*trnK*), and *BII* (*rpoB*) (Nettel and others 2009). These five loci were amplified for a total of 505 individuals from the 43 populations sampled. Because of haplotype monomorphism in populations, we amplified fewer individuals than were used in nuclear microsatellite analyses. PCR conditions of the cpDNA microsatellite loci, as well as sequencing procedures, are described elsewhere (Nettel and Dodd 2007). Amplifications were electrophoresed on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA).

Nuclear DNA

Eight nuclear microsatellite loci developed for tanoak (LD1, LD3, LD5, LD7, LD8, LD10, LD12, LD14, Morris and Dodd 2006) were PCR amplified using a fluorescent labeled primer. Primers were multiplexed in five different groups: i) LD1, LD3, LD7, LD10, LD14; ii) LD5; iii) LD12; and iv) LD8. The PCR cocktail contained 1X PCR Buffer, 2.0mM MgCl2, 0.2 mM of each dNTP, 250nM of each reverse primer, 250nM of each fluorescently labeled (FAM or HEX) forward primer, 1 unit of Amplitaq Polymerase (Invitrogen, Carlsbad, CA) and approximately 5 ng template DNA in a 20 µl reaction. Forward and reverse primers for the locus LD3

were run at a final concentration of 400nM, exclusively. Touchdown PCR cycling conditions for all loci were as follows: one cycle at 95 ˚C for 10 minutes followed by 20 cycles of 45 seconds at 94 ˚C, 45 seconds at 58 ˚C (lowering 0.5 ˚C each cycle), and 45 seconds at 72 ˚C. The final amplification step consisted of 20 cycles of 45 seconds at 94 ˚C, 45 seconds at 48 ˚C, and 45 seconds at 72 ˚C and was followed by a final extension step at 72 °C for 45 minutes. All reactions were performed on a Techne (UK) Flexigene thermocycler. We mixed 0.75 µl of PCR product with 8 µl of formamide and 0.5 µl of 500 LIZ size standard (Applied Biosystems, Foster City, CA) and we electrophoresed this cocktail on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA). We used GENESCAN 3.7 and GENOTYPER 3.7 (Applied Biosystems, Foster City, CA) to analyze ABI results. We sequenced Loci LD3 and LD5 because they showed odd-sized series in fragment analyses. The sequences showed that indels within the flanking regions were responsible for the different size series and so the fragment sizes were adjusted appropriately.

Table 1—Location of sampling sites

Nearest location	County/State	Lat.	Long.	Sample #	Subspp.
Santa Barbara	Santa Barbara/Ca	34.50	-119.82	20	*densiflorus*
Lompoc	Santa Barbara/Ca	34.58	-120.50	24	*densiflorus*
Nacimiento	Monterey/Ca	35.81	-120.75	41	*densiflorus*
Pfeiffer Big Sur	Monterey/Ca	36.25	-121.78	17	*densiflorus*
Palo Colorado	Monterey/Ca	36.39	-121.90	32	*densiflorus*
Soquel	Santa Cruz, Ca	37.05	-121.85	57	*densiflorus*
S. F. Peninsula	San Mateo/Ca	37.40	-122.28	31	*densiflorus*
Point Reyes	Marin/Ca	38.06	-122.80	33	*densiflorus*
Forestville	Sonoma/Ca	38.47	-122.90	10	*densiflorus*
Cazadero	Sonoma/Ca	38.49	-123.06	10	*densiflorus*
Salt Point	Sonoma/Ca	38.56	-123.32	10	*densiflorus*
Gualala	Sonoma/Ca	39.35	-123.52	10	*densiflorus*
Fort Bragg	Mendocino/Ca	39.43	-123.80	7	*densiflorus*
Jackson SF	Mendocino/Ca	39.35	-123.61	24	*densiflorus*
Sinkyone	Mendocino/Ca	39.78	-12.83	20	*densiflorus*
Hickey	Mendocino/Ca	39.88	-123.73	20	*densiflorus*
King Range	Humboldt/Ca	40.16	-124.08	15	*densiflorus*
Weott	Humboldt/Ca	40.34	-123.95	9	*densiflorus*
Hacketsville	Humboldt/Ca	40.50	-124.16	18	*densiflorus*
Grizzly Creek	Humboldt/Ca	40.84	-123.91	20	*densiflorus*
Redwood Creek	Humboldt/Ca	40.85	-123.81	21	*densiflorus*
Korbel	Humboldt/Ca	40.85	-123.92	20	*densiflorus*
Fickle Hill	Humboldt/Ca	40.85	-123.82	20	*densiflorus*
PrairieCrk	Del Norte/Ca	41.45	-124.02	20	*densiflorus*
Jedediah	Del Norte/Ca	41.81	-124.08	20	*densiflorus*
Gasquet	Del Norte/Ca	41.88	-123.85	20	*densiflorus*
Gasquet	Del Norte/Ca	41.89	-123.99	13	*echinoides*
Elk Valley	Del Norte/Ca	41.99	-123.72	17	*densiflorus*
O'brien	Josephine/Or	42.07	-123.72	25	*densiflorus*
Brookings	Curry/Or	42.09	-124.31	21	*densiflorus*
Williams	Josephine/Or	42.26	-123.38	16	*densiflorus*
Sebastien	Curry/Or	42.32	-124.40	20	*densiflorus*
Merlin	Douglas/Or	42.52	-123.62	20	*densiflorus*
Agness	Curry/Or	42.53	-124.22	20	*densiflorus*
Glendale	Douglas/Or	42.73	-123.53	20	*densiflorus*
Marial	Curry/Or	42.81	-124.21	21	*densiflorus*
Powers	Coos/Or	42.93	-124.08	21	*densiflorus*
Dora	Coos/Or	43.17	-124.02	21	*densiflorus*
Blodgett	El Dorado/Ca	38.73	-120.75	54	*densiflorus*
Plumas	Butte/Ca	39.93	-121.58	10	*densiflorus*
Weaverville	Trinity/Ca	40.69	-122.93	17	*densiflorus*

Data Analysis

We checked data quality for scoring errors in nuclear and cpDNA microsatellites using MICRO-CHECKER (Oosterhout and others 2004). We used the same program to estimate the probability of the presence of null alleles in nuclear loci.

Chloroplast microsatellite alleles were combined into haplotypes, where each amplified locus was considered an independent, but linked, character inherited as a unity.

To ascertain reliability of the estimations based on nuclear microsatellites, we tested

deviations from Hardy-Weinberg (HW) equilibrium within each population by the inbreeding fixation index, *F*IS, with the software FSTAT ver. 2.9.3.2 (Goudet 2002). HW equilibrium could not be rejected for any of the populations. We estimated allelic richness with the rarefaction method (*R*t) and expected heterozygosity (*H*e) with FSTAT in order to analyze the level of within population genetic diversity. We used BAPS 5.2 (Corander and others 2008) to infer population structure from the nuclear microsatellite diversity. We inferred population division (number of *K* populations) by performing 20 independent runs of each *K* (*K*= 1 to *K*= 40).

Results

Four major, and two rare chloroplast DNA haplotypes were detected (fig. 1). The four major haplotypes included: 1. a central and northern coastal California haplotype (B) with the northern limit near Korbel, Humboldt County, California; 2. an extreme northern California and Oregon haplotype (D) with a southern limit at Hoopa, Humboldt County, California; 3. a Sierra Nevada haplotype (E) found in populations from El Dorado and Butte Counties; and 4. a Klamath haplotype (F) from east of Weaverville in Trinity County. The rare haplotypes (A and C) occurred as four individuals (three from Palo Colorado and one from Big Sur) and a single individual (from Cazadero) respectively. With the exception of populations with the two rare haplotypes, we detected a mixture of haplotypes in only one population - haplotypes B and D in approximately equal proportions in a population between Korbel and Hoopa, Humboldt County. Interestingly, the northernmost population from Dora, Coos County, Oregon had the B haplotype characteristic of central and northern coastal California populations, suggesting a possible non-indigenous source.

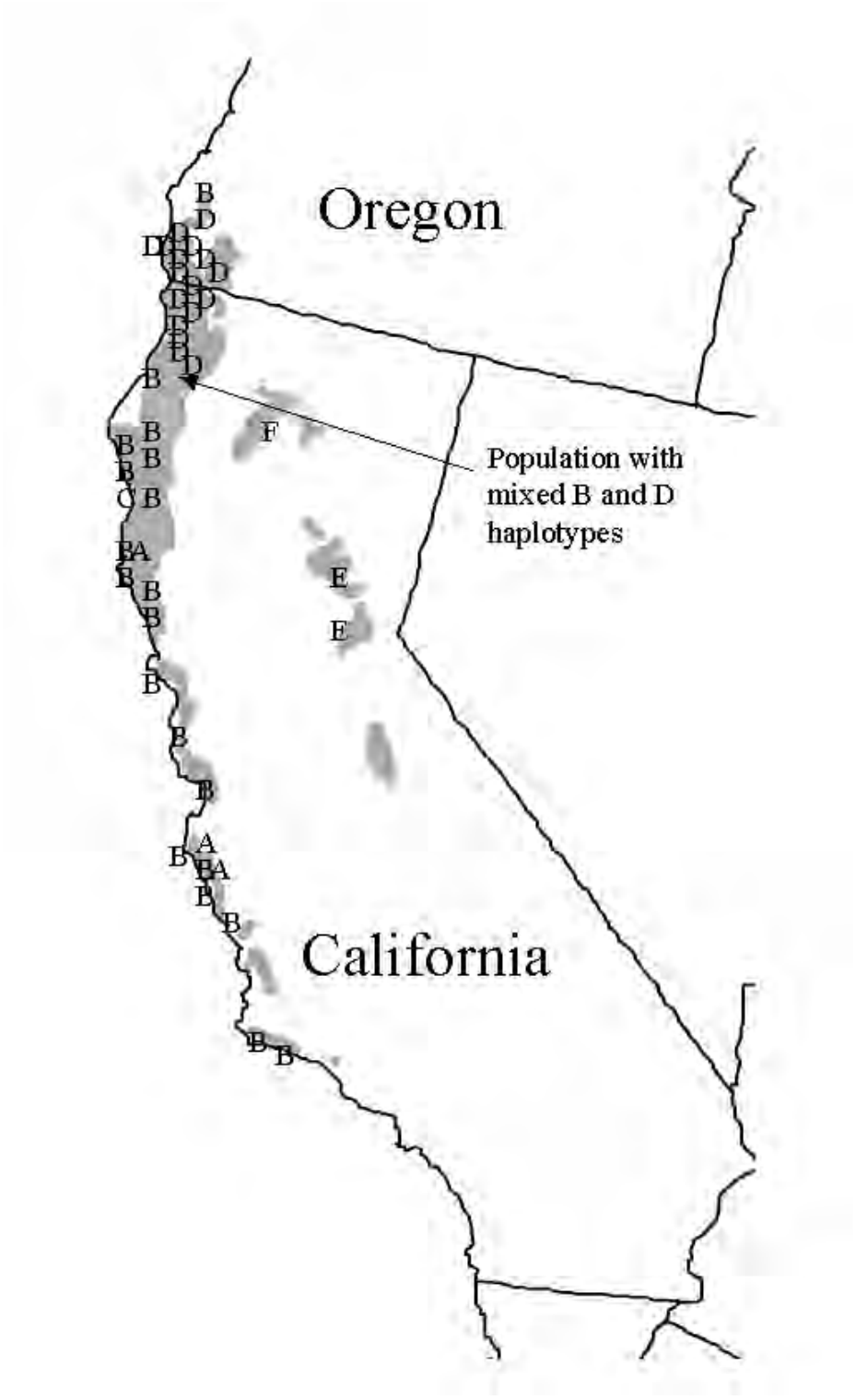

Figure 1—Distribution of chloroplast haplotypes. Four major haplotypes (B,D,E,F) and two rare haplotypes (A,C). Shading shows the distribution of tanoak from Tappeiner and others 1990.

Based on nuclear microsatellite diversity, the 905 individuals were assigned to 11

clusters with a posterior probability of 0.99. The partition of clusters is shown as a Voronoi tessellation in fig. 2. Significant breaks occurred: 1. between Hoopa and Korbel, consistent with the chloroplast haplotype lineage divergence; 2. near San Francisco between Point Reyes and the mid-San Francisco Peninsula; and 3. between coastal and interior (Weaverville, El Dorado, and Butte) populations. The three southernmost populations (Nacimiento, Lompoc, and Santa Barbara) and the three interior populations formed single population clusters. A single population of dwarf tanoak formed its own cluster suggesting lack of gene flow between it and nearby sources of the tree form. An unrooted tree based on Nei's genetic distances indicated major divergence between the two subspecies (fig. *2*). Interior populations of ssp. *densiflorus* were widely divergent from coastal populations. Among the latter, southern California populations from Nacimiento, Lompoc, and Santa Barbara were well differentiated, but remaining populations from central and northern California and Oregon showed relatively low levels of differentiation.

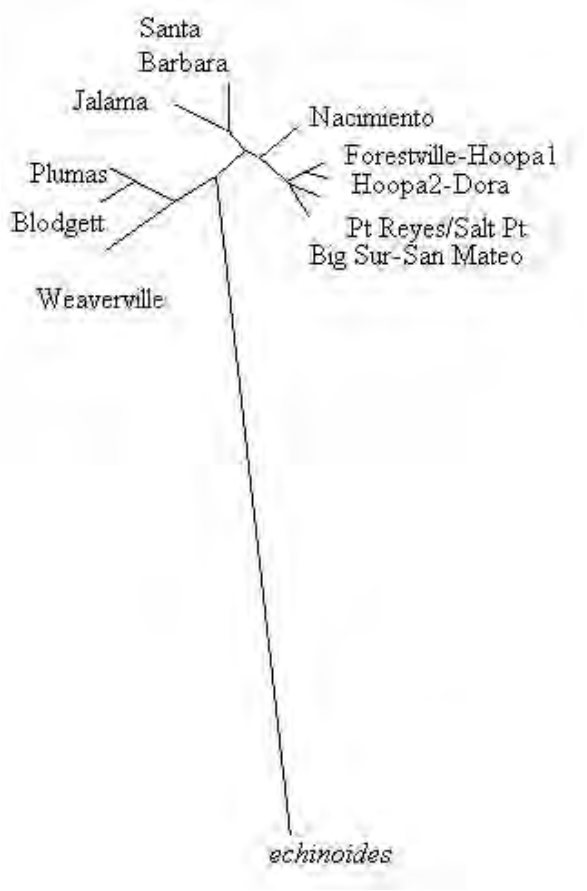

Figure 2—Unrooted tree based on Nei's genetic distances.

Genetic diversity, measured as allelic diversity, or as expected heterozygosity, increased significantly with latitude (fig. *3*). Using the same groupings detected by BAPS, we tested for differences in genetic diversity among selected groups (table 2). Overall, genetic diversity estimates were significantly different among groups. In pairwise comparisons, southernmost populations had significantly lower diversity than central coast populations and interior populations (Weaverville, Plumas, and Blodgett) had significantly higher diversity than all other populations.

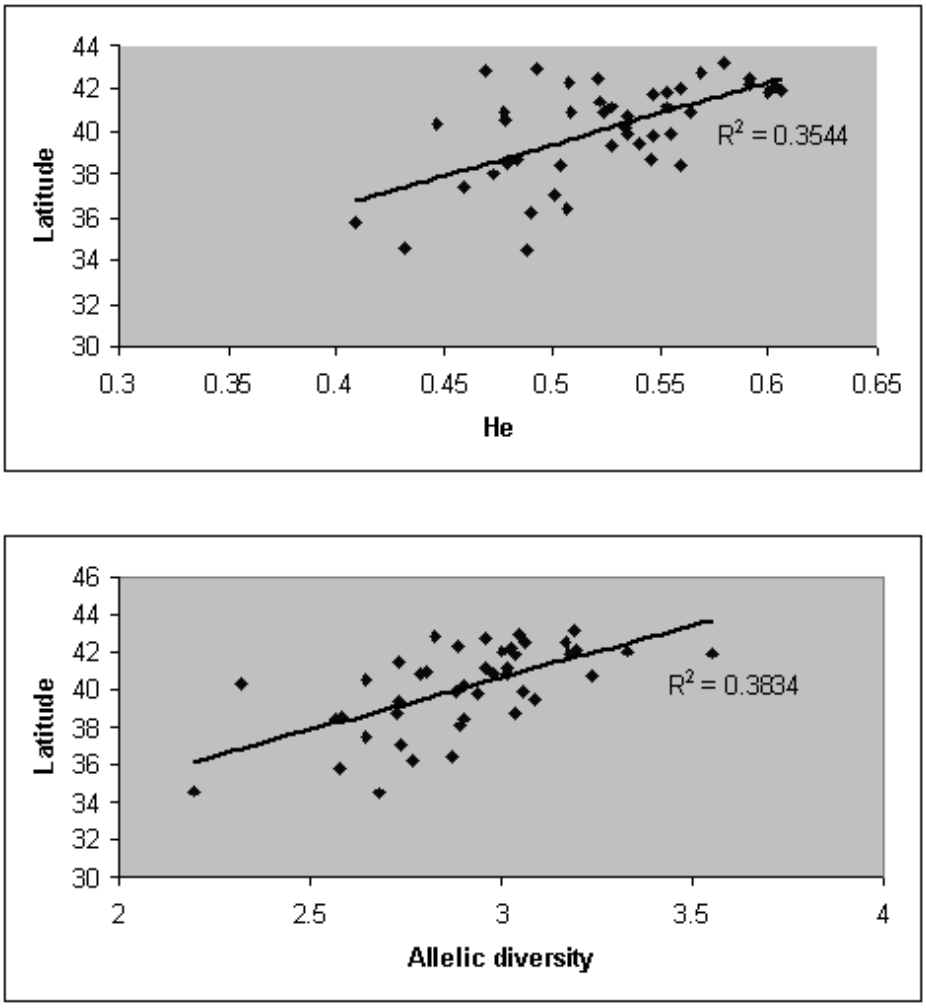

Figure 3—Plots of population genetic diversity, measured as expected heterozygosity (H_e) and allelic diversity, by latitude.

Table 2—Genetic diversity measured by allelic richness (*A*) and expected heterozygosity (H_S) for major clusters of populations of tanoak

	Group1	Group2	Group3	Group4	Group5
A	2.49	2.76*	2.82	3.00	3.11*
H_S	0.44	0.49	0.52	0.55	0.54

Groups 1 – 4 reflect increase in latitude, Group 5 is interior populations. Group1 – Nacimiento, Lompoc, Santa Barbara; Group 2 – Pfeiffer Big Sur to San Francisco Peninsula; Group 3 – Point Reyes to Korbel; Group 4 – Hoopa to Dora; Group 5 – Weaverville, Plumas, and Blodgett. Overall, two-sided probabilities after 10,000 permutations were $A = 0.006$ and $H_S = 0.005$. Asterisks indicate populations that showed significantly greater diversity than the group to the left in the table (one-tailed tests). All tests were carried out in FSTAT (Goudet 2002).

Discussion

Disease epidemics in forest ecosystems can have devastating consequences when the susceptible host is a keystone species. A cascade of biotic and abiotic effects can lead to transformation of the ecosystem, imposing permanent ecological, social and economic costs. Screening for resistance and protection of populations of conservation interest are two priorities in the early stages of the disease outbreaks. In wild ecosystems, the structure of genetic diversity in the threatened host is rarely known and must be one of the first avenues to be addressed for resource management. Screening for resistance is expensive and time-consuming and can be helped by knowledge of host genetic structure that might indicate the optimal sampling strategy. Conservation is aimed at maintaining existing evolutionary potential through identification of population lineages that may have unique adaptive potential (Moritz 1994, Crandall and others 2000). The recent SOD epidemic has indications of becoming a major transforming influence in the coastal forests and woodlands of California and Oregon (Barrett and others 2006, Meentemeyer and others 2004) and currently tanoak is the host that has suffered greatest mortality. Screening of tanoak for resistance has begun (Hayden and others, unpublished data), but the rationale for selecting populations for screening is hampered by lack of knowledge of genetic structure of this host species. This paper reports on variation in genetic diversity over an extensive range-wide sampling scheme.

Chloroplast haplotype diversity in tanoak is low, as expected for plants with heavy seeds and no specialized systems for long distance dispersal. The partition of haplotypes indicates the geographic limits to colonization by seed, and is therefore an excellent conservative estimate of seed zones based on the likely expansion of populations since the last glacial maximum (LGM). It is likely that the distribution of tanoak was restricted during the LGM, with some populations occupying sites where climatic conditions remained suitable. Four major haplotypes were detected indicating interior lineages in the Sierra Nevada and the Klamath Mountains, a coastal California lineage and a northern California-Oregon lineage. The distribution of tanoak today is discontinuous from the Sierra Nevada through the Klamath Mountains to coastal California, and so seed dispersal across these breaks is unlikely. However, the divergence of chloroplast haplotypes near to Arcata, Humboldt County, separating central and northern coastal California from extreme north coastal California and Oregon, is interesting. The significance of this break is supported by divergence in nuclear microsatellite diversity, indicating that gene flow by pollen has not been sufficiently invasive to break down the divergence between these two coastal chloroplast lineages. This would suggest that the northern and southern

lineages have only relatively recently met, or that there are partial barriers to mating. The latter could be realized by differences in average flowering times, a hypothesis that deserves further investigation.

Nuclear microsatellite divergence supports the lineages detected by chloroplast DNA, but also indicates some further differentiation among populations. Most significantly, analysis of nuclear DNA indicates a break between populations north and south of the San Francisco Bay and divergence among the three southernmost populations at Nacimiento, Lompoc, and Santa Barbara. In the southern range of tanoak, populations are dispersed and commonly relatively small. It is likely that lack of gene flow among these populations has led to differentiation through genetic drift. These southernmost populations exhibited the lowest levels of genetic diversity as would be expected if they were diverging through genetic drift. Levels of genetic diversity increase northwards, but interestingly the highest levels of diversity are in the Sierra Nevada/Klamath populations.

Tanoak Conservation

Many coastal tanoak populations have been heavily affected by the SOD epidemic in the last decade; mortality rates of more than 60 percent have been reported (Maloney and others 2005) and an exponential increase of mortality has been detected over a five-year period (Cobb and others 2008). Furthermore, the predicted trend for climate warming (IPCC 2001) is likely to affect tanoak adversely. The loss of tanoak trees throughout the different ecosystems where it is a major component will result in serious consequences for northern California and southern Oregon landscapes. Effects could be devastating, including a loss of wildlife that depend on acorns, a raise of fuel loads that would result in more extreme wildfires, and an increase in the risk of soil erosion in coastal ecosystems. Conservation efforts for habitat restoration and to preserve the evolutionary potential of this species are likely to be needed in the near future. Our study sheds light on the partition of genetic diversity at putatively neutral loci in both the nuclear and chloroplast genomes. Genetic variation and genetic differentiation throughout the range has to be taken into account to determine conservation priorities, seed sources for long-term preservation, and reforestation efforts.

Based on our chloroplast and nuclear DNA data, we identify six regions that should be considered as breeding groups that should be included in any resistance screening and should be represented in conservation management. We use the term breeding groups rather than seed zones because we cannot infer growth performance from our neutral DNA markers. 1. Extreme southern populations that in our sampling include Nacimiento, Lompoc, and Santa Barbara - These are identified as relatively depauperate genetically and should be considered as important in a conservation context. 2. Central coastal California from Pfeiffer Big Sur north to the San Francisco Peninsula - This region includes populations that have recently been ravaged by SOD disease and may therefore offer the opportunity for identifying tolerant individuals. 3. Northern coastal California from north of the San Francisco Bay to Arcata in Humboldt County - Some of the southern populations in this range have also been devastated by disease and will offer the possibility of identifying tolerant individuals. 4. Extreme north coastal California and Oregon - This is an area of extensive tanoak woodlands. Genetic diversity is relatively high and may therefore offer opportunities for finding diverse genotypes that may include resistant/tolerant traits. 5. Klamath

region - Our sampling in this region is relatively low, although ssp. *echinoides* has been sampled. This is an important region for conservation and screening and requires further investigation. 6. Sierra Nevada - The high genetic diversity of interior populations, relatively deep divergence from coastal populations, and lack of disease in this region make it an important component of resistant screening.

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Tanoak Resistance: Can it be Used to Sustain Populations?[1]

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Abstract

Tanoak (*Lithocarpus densiflorus*) trees are among *Phytophthora ramorum*'s most susceptible hosts. Extensive mortality in this species has led researchers to question whether selective breeding for resistance can be used to sustain populations; the answer depends on the extent and heritability of pathogen resistance within the host. Consequently, we have undertaken a multi-year common garden study of resistance to *P. ramorum* in tanoak seedlings grown from acorns collected by collaborators at sites in California and southern Oregon.

We have sown 12,650 acorns from nine unique sites in a common garden since 2006. The resulting seedlings have been assayed for resistance to *P. ramorum* by both detached leaf inoculations using plugs of mycelia as the infective agent, and seedling tip inoculations using a zoospore suspension. Both assays revealed variable resistance with significant heritability.

In addition to the laboratory assays, a subset of 800 seedlings from 50 different family groups were planted in a heavily infested, forested site in Monterey County, California. These seedlings are currently being monitored to determine whether there is a correlation between family-level variation in resistance in the laboratory setting to survivorship in the field. After 1 year, the survival rate was 82.5 percent, with no discernable effect of family; however, there were positive identifications of natural infection by *P. ramorum*.

Together, the data from these studies provide not only background knowledge crucial to predicting the evolutionary and ecological outcomes of the *P. ramorum* epidemic in tanoak populations, but also for ascertaining any potential for genetic resistance in tanoak to be used as a management tool.

Introduction

Tanoak (*Lithocarpus densiflorus*) trees are among *Phytophthora ramorum*'s most susceptible hosts. Incidence rates have been measured at 30 to 90 percent in infested areas (Maloney and others 2005, McPherson and others 2005, Meentemeyer and others 2008), and the median survival time for a symptomatic tree has been estimated to be 2.9 to 8.7 years (McPherson and others 2005). The high incidences coupled with high mortality have led researchers to question whether selective breeding for resistance can be used to sustain populations. Assessing the feasibility of a breeding program requires a great deal of background information, most of which is lacking

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for tanoaks. First and foremost, there must be heritable variation for pathogen resistance within the host in order for there to be any evolution of greater disease resistance in the tree population, whether by natural or artificial selection (Parker and Gilbert 2004, Simms 1996). Furthermore, for this variation to be used in management, it must be able to be appropriately assayed (Carson and Carson 1989, Sniezko 2006). In order to address these primary questions, we have undertaken a multi-year common garden study of resistance to *P. ramorum* in tanoak seedlings grown from acorns collected by collaborators at sites in California and southern Oregon.

Methods and Materials

Since 2006, we have sown 12,650 acorns from nine unique sites in pots in a common garden at the Oxford Tract Research Greenhouses in Berkeley, California. Collections have been made yearly, beginning with acorns provided by R. Dodd (University of California) and C. Roessler (Midpeninsula Regional Open Space District) from five sites in 2006, with sites ranging from Monterey County, California in the south to Curry County, Oregon, in the north, and El Dorado County, California (Blodgett Forest Research Station, in the western Sierra Nevada foothills) in the east. In 2007, the Blodgett collection was repeated (R. Dodd) because of poor growth the prior year. Further collections were made in 2008; these seedlings will be tested for resistance in future trials.

Resistance Assays, Laboratory

At 1 year of age, the 2006 and 2007 seedling cohorts were each assayed for resistance to *P. ramorum* by detached leaf inoculations using plugs of mycelia (isolate Pr52, CBS110537, ATCC MYA-2436) as the infective agent. Briefly, agar plugs were set on the freshly cut petiole of detached leaves (two to four replicate leaves per seedling), and incubated in moist chambers at 18 to 20 °C for 2 weeks. Replicates from each tree were incubated in different chambers. Lesions extended up the midrib, and lesion and leaf lengths were measured with Assess (APS Press, St. Paul, MN); the natural-log transformed ratio of lesion to leaf length was used for analyses. The 1033 seedlings from 71 families in the 2006 cohort were assayed in February 2008, and 448 seedlings from 22 families in the 2007 cohort were assayed in November 2008.

The 2007 cohort was assayed using a whole-seedling tip inoculation in December 2008. A suspension of Pr52 zoospores (1×10^4 spores/ml) was dropped onto a wax cup wrapped around the wounded seedling tip. Lesion length was measured monthly, as were the development of symptomatic leaves and mortality. The data presented here were taken 4 months after inoculation; data collection will continue for up to 1 year.

Variances in lesion lengths were analyzed by mixed-model, nested ANOVA. For detached leaves, the model was:

$y_{ijklm} = \mu + C_i + S_j + P_{k(j)} + T_{l(k,j)} + E_{ijklm}$

where y_{ijklmn} is the predicted lesion value for m^{th} observation of the l^{th} seedling of the k^{th} parent from the j^{th} site, μ is the grand mean, C is the i^{th} incubation chamber, S_j is the source site, $P_{k(j)}$ is the parent, nested within site, $T_{l(k,j)}$ is seedling, nested within

parent and site, and E_{ijklm} is the residual variation. Site, parent, and seedling were modeled as random effects; chamber was fixed. The 2007 cohort included only a single source site, so site was omitted from that model.

Lesions resulting from seedling tip inoculations were modeled as:
$y_{kl} = \mu + P_k + E_{kl}$
where y_{kl} is the predicted lesion value for the l^{th} seedling from the k^{th} parent family, and E_{kl} is the residual; parent was a random effect. Seedling stem height, diameter, and block were originally included in the model as fixed effects, but were non-significant and were removed.

Because these open-pollinated families are expected to contain a mixture of full- and half-siblings, narrow-sense heritability of lesion size was calculated as $h^2 = 3V_P/V_T$, where V_P/V_T is the proportion of total variance due to shared parent.

Resistance Assays, Field

In addition to the laboratory assays, 800 seedlings from 50 families in the 2006 cohort were planted out in 10 different plots in two *P. ramorum*-infested canyons in the Santa Lucia Preserve, Carmel Valley, California in January 2008. Four seedlings were randomly placed in each of 4 different plots, for a total of 16 seedlings planted per family. These seedlings will be followed for at least 3 years to track natural infection rates and symptom development. Data were taken quarterly, including growth, number of symptomatic leaves, stem lesions, and dieback; results reported here are from the first year of monitoring.

Small mammal herbivory, consistent with rabbits, was observed at half of the plots, with 26 percent of all seedling severely herbivorized. As of May 2009, all seedlings were individually caged, and 30 percent of the herbivorized plants showed regrowth.

Results

Resistance Assays, Laboratory

Maternal family contributed significantly to variance in resistance, measured by lesion size, in all assays (table 1, table 2). There was a trend toward greater heritability of resistance to lesion expansion 4 months after seedling tip inoculation ($h^2 = 0.51$, 95 percent CI 0.26-1.52) than in either detached leaf assay (2006 $h^2 = 0.14$, 95 percent CI 0.04-0.26; 2007 $h^2 = 0.14$, 95 percent CI 0.05-0.46); notably, all confidence intervals overlap. There was no significant geographic trend in resistance by the detached-leaf assay.

In detached leaves, shared-parent family mean lesion lengths ranged from 22 to 44 percent of the leaf length. After tip inoculation, family mean lesion lengths ranged from 3.4 cm to 7.5 cm, and infection resistance (percentage of seedlings with no apparent symptoms after inoculation, as of May 2009) ranged from 0 to 22 percent. Infection in detached leaves was 100 percent; there was no infection resistance by detached-leaf assay.

As of April 2009, 4 months after inoculation, overall survivorship of tip-inoculated seedlings was 86.5 percent. Mortality was exponentially associated with median family lesion length (fig. 1, $P < 0.0001$, exponential fit $R^2 = 0.76$).

Table 1—Analysis of variance: random effects on lesion as proportion of leaf length in detached leaf inoculation assays. Parent refers to open-pollinated maternal seed parent

Random Effect	Variance Ratio	Variance Component (95% CI)	% Total
2006 cohort			
Site	0.167	0.011 (0.003-0.180)	10.78
Parent(Site)	0.071	0.005 (0.003-0.009)	4.59
Seedling (Site, Parent)	0.312	0.016 (0.016-0.025)	20.13
Residual		0.064	
Total		0.100	
2007 cohort			
Parent	0.075	0.003 (0.001-0.010)	4.73
Seedling (Parent)	0.521	0.021 (0.017-0.027)	32.63
Residual		0.041	62.64
Total		0.065	

Table 2—Analysis of variance: random effects on lesion length in seedling tip inoculation assay

Random Effect	Variance Ratio	Variance Component (95% CI)	% Total
Parent	0.205	2.28 (1.123-6.788)	17.01
Residual		11.125	83.00
Total		13.404	

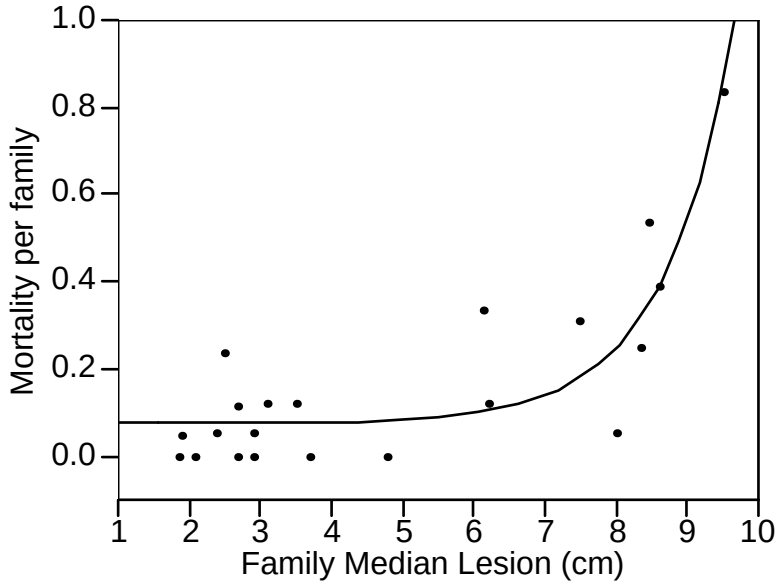

Figure 1—Association between mortality and family median lesion length in tanoak seedlings tip-inoculated with *P. ramorum*. Mortality = 0.075 + 0.000058 Exp(Median Lesion), $P < 0.0001$, $R^2 = 0.76$.

Resistance Assays, Field

At 6 months after planting, *P. ramorum* infection was confirmed in 26 seedlings using TaqMan detection (Hayden and others 2006). After 1 year, the pathogen was identified in an 11 additional seedlings using morphological identification of isolates on selective media, with molecular diagnostics ongoing. The 1-year survival rate was 82.5 percent, with no discernable effect of family.

Conclusions

The data from these studies help to provide the background knowledge crucial both to predicting the evolutionary and ecological outcomes of the *P. ramorum* epidemic in tanoak populations, and for ascertaining any potential for genetic resistance in tanoak to be used as a management tool. The inoculation assays revealed heritable genetic variation in resistance to *P. ramorum* in tanoak seedlings in a laboratory setting, and provide two methods with which to identify candidate families for further study. These assays each have advantages: while the tip inoculation most closely mimics natural infections, it is destructive and therefore not replicable. The detached leaf inoculation is replicable, but may have a smaller genetic component. The assays likely measure different resistance mechanisms; both may be useful for predicting disease outcomes. Tip-inoculated seedlings will continue to be monitored; differences between seedlings and families may be more apparent after a longer period of pathogen growth.

Further acorn collections are planned. While the first collections were designed to sample randomly in order to include as much natural variation as possible, collections from 2008 onwards are targeted towards individuals that may be expected to have greater than average resistance, such as surviving trees in areas of high mortality. The seedlings from these collections will be assayed by both detached-leaf and seedling tip assays, with an emphasis on tip inoculations. Seedlings from the 2006 collection were tip-inoculated in May 2009; the results of this assay will be compared with those of both detached-leaf inoculations and field studies.

There was infection of seedlings under natural conditions in the first year of the field resistance study; we expect symptom development to intensify in the second autumn. This field component will provide a crucial avenue to validate applicability of lab studies to real-world survivorship. The presence of natural infection demonstrates the utility of the study design, and our experience will allow refinement of the technique for future use. Moreover, the establishment of a common garden population establishes a long-term resource that may continue to be used for genetic study.

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Relationship Between Resistance to *Phytophthora ramorum* and Constitutive Phenolic Chemistry in Coast Live Oaks and Northern Red Oaks[1]

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Abstract

Phytophthora ramorum causes lethal canker diseases and extensive mortality in coast live oak (CLO) (*Quercus agrifolia*) and tanoak (*Lithocarpus densiflorus*). No practical controls are available for this disease in non-urban environments. Therefore, characterization of natural resistance is highly desirable. Variation in resistance to *P. ramorum* has been observed in CLO in both naturally infected trees and controlled inoculation trials. The persistence of asymptomatic CLOs in naturally infested disease progression plots established in 2000 has been reported (McPherson and others 2005; McPherson and others, unpublished). Around a third of CLOs in a population directly inoculated with the pathogen failed to develop symptoms or appeared to recover following initial symptom development (McPherson, unpublished). Previous studies suggested that phloem phenolic chemistry may play a role in induced defense responses to *P. ramorum* in CLO (Ockels and others 2007). However, in those studies, a relationship was not established between phenolic defense responses and actual resistance, and constitutive phenolic levels may also play a role in resistance, tolerance, or mitigation of initial infection.

The escape of *P. ramorum* into native forests outside of its current range is also highly feared. *Quercus* spp. are dominant throughout eastern North American forests and are extremely important from both ecological and economic standpoints. Laboratory inoculations have demonstrated susceptibility to *P. ramorum* in many eastern tree species, with northern red oak, *Quercus rubra* (NRO), being the third most susceptible species tested (Tooley and Kyde 2007). The pathogen has also been isolated from bleeding cankers on landscape NROs in The Netherlands (Brasier and others 2004), but there is no information concerning variation in susceptibility within the species.

Phenolics are an extremely diverse class of highly bioactive, and in many cases, highly toxic secondary metabolites. Accumulation (both constitutive and induced) of certain phenolics has been implicated in defense strategies, particularly in conifers, where they have been more intensively studied, but in a few angiospermous species as well (reviewed by Witzell and Martin 2008).

Here we describe investigations aiming to elucidate the role of constitutive phenolics in resistance by quantifying the relationship between concentrations of individual and total phenolics (quantified by HPLC analysis) to actual resistance in CLO and NRO. Our long-term

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goal is to identify easily screenable biomarkers of resistance. Three experiments were conducted towards this goal. In Experiment one, we used cohorts of CLOs that had previously been characterized as relatively resistant (R) or susceptible (S) (D. Huberli, personal communication). Constitutive (pre-inoculation) phenolics were extracted from branches harvested from R and S trees on three different dates (October 2007, April 2008, November 2008). Additional branches from the same trees were inoculated in the greenhouse at the time of phloem sampling to confirm relative resistance. In Experiment two, concentrations and variation in phenolics of CLOs exhibiting apparent field resistance, in other words, that remained asymptomatic (PR: putatively resistant) under high disease pressure in the field and/or after artificial inoculation during 5 to 8 years of continuous observation (McPherson and others 2005, 2008) were compared to symptomatic (S: susceptible) trees and trees that had shown symptoms at one time and then recovered (PS: previously symptomatic). We tested two cohorts of remnant CLOs, both in stands with elevated infection levels, one representing remnant trees in an infection center with high mortality (China Camp) and the other (Nike) subjected to artificial inoculations in 2002. In Experiment three, 10 half-sib families of 1-year-old NRO were inoculated and their relative resistance evaluated against concentrations of constitutive phenolics.

In Experiment one, there was a significant effect of date on average lesion length ($F_{2, 36}$ = 3.52, P = 0.040). Dodd and others (2005) observed a similar seasonal trend. In October 2007, trees in the R group had significantly shorter lesions than trees in the S group, confirming their *a priori* rankings, and though R and S trees did not differ in concentrations of any phenolics tested, there was a significant negative correlation between average within-tree concentrations of a tyrosol derivative and lesion lengths (Spearman's $\rho = -0.733$, P = 0.012). In April 2008 and November 2008 lesions in R and S trees were not significantly different, suggesting that branch bioassays under controlled conditions did not provide a reliable evaluation of relative field resistance in our study. However, Dodd and others (2008) showed consistency in relative susceptibility rankings during multiple springtime inoculations. Because our cohort of trees did not display consistent levels of relative resistance in the second and third trials, we cannot compare trends in phenolic chemistry, and substantiate the October 2007 results.

In Experiment two, there were no significant differences between PR, PS, and S for any individual compounds or for total phenolics in the China Camp cohort. In the Nike study, total phenolics did not differ between tree categories, but levels of a tyrosol derivative and ellagic acid varied significantly between PR, PS, and/or S. Biochemical activity of ellagic acid against *P. ramorum* is unknown, and the tyrosol derivative will need to be chemically characterized before activity can be postulated. Nevertheless, Ockels and others (2007) described a strong, dose-dependent inhibitory effect of tyrosol on *P. ramorum* and other *Phytophthora* spp. *in vitro,* and antifungal activity of tyrosol has been described elsewhere (Slininger and others 2004, Baidez and others 2006). The UV spectra of tyrosol and the unknown derivatives were identical, and they varied only by their elution times. Results from the Nike study showed that the constitutive concentration of certain, perhaps critical, phenolics were higher in PR and PS trees. This pattern becomes particularly interesting when trees in PR and PS are conceptualized as "more resistant" than currently symptomatic trees.

In Experiment three, NROs were screened for familial variation in phenolic chemistry and susceptibility to *P. ramorum,* and the relationship between peak area of individual phenolics and lesion length was examined. We found up to five-fold variation in mean lesion lengths between families. Although there was not an overall effect of family on lesion length (Kruskal-Wallis, $\chi^2 = 13.39$, P = 0.146), average lesion length was significantly different between some families in pair-wise comparisons, suggesting there may be useful variation in susceptibility within the species. We screened individual compounds (HPLC peaks) for a possible defensive role by correlating peak area with lesion length. Those compounds correlating negatively with lesion length may be involved in fungal growth inhibition and thus resistance. Significant, negative correlations were found for six peaks. We also tested each

peak for a family effect. A significant family effect may indicate that regulation or expression of the compound in question is heritable. Phenolics showing both negative correlations with lesion length and significant family effects are particularly good candidates for use as biomarkers in breeding resistant NROs. Four peaks met both of these requirements, but will need to be chemically characterized and tested in bioassays to determine potential biological activities.

Some of the inconsistency in our CLO results may be due to our inability to identify, post epidemic, trees that are truly resistant and susceptible to *P. ramorum*. The fact that we were unable to obtain representative samples of the most susceptible CLOs prior to their infection and death makes comparing chemical defenses between R and S trees problematic. This issue may only be resolved by banking information on constitutive phenolics from large numbers of trees prior to infestation, with comparisons made following death of the most susceptible trees.

In spite of these limitations, it does appear that production of tyrosol derivatives is upregulated in the more resistant CLOs examined. Taken together, significantly higher levels of ellagic acid and a tyrosol derivative observed in the Nike trees (the most reliable of the two field studies), the negative correlation between a different tyrosol derivative and lesion length in October 2007 in Experiment one (the only trial where R and S trees could be statistically separated), and the strong *in vitro* anti-*Phytophthora* activity of tyrosol (Ockels and others 2007) suggest that these compounds may be especially good candidates for further examination as potential biomarkers for resistance of CLO to *P. ramorum*. Similarly, four unidentified phenolic compounds in NRO were identified as biomarker candidates based on lesion length and family effect, but chemical characterization of these compounds must occur before their potential defense role can be evaluated. Studies on both remnant CLO and NRO must be repeated to validate results, and data from winter and spring dates may be especially ecologically relevant, given that this is when natural infection appears to take place in coastal California forests (Rizzo and others 2005).

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Variation in Density and Diversity of Species of *Phytophthora* in Two Forest Stream Networks[1]

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

Abstract

Monitoring occurrence and distribution of *Phytophthora* species, including *Phytophthora ramorum*, in forest ecosystems can be achieved in several ways including sampling symptomatic plants, infested soils, and infested streams. Collecting plant and soil samples can be laborious and time consuming due to the distance surveyors need to travel. Not all forests are available for survey because of limited accessibility and stand density. Species of *Phytophthora* are well adapted to aquatic habitats and more diverse populations of *Phytophthora* spp. are found in forest streams than in nearby soils and symptomatic riparian plants. The current protocol for the National *P. ramorum* Early Detection Survey, conducted by the U.S. Department of Agriculture Forest Service (USDA FS), adopted sampling forest streams using baiting and filtration procedures. One assumption being made in these monitoring efforts is that species present in a stream network are representative of those present in the land area drained by that network. Therefore, careful selection of stream networks is essential to optimize the sampling effort with limited available resources, and strategic selection of sample sites within a stream network should maximize detection of the species of *Phtyophthora* present. A stream network in a natural ecosystem consists of a main stream, its tributaries, and a drainage point of the main stream. In this study, our hypothesis was that the occurrence and diversity of *Phytophthora* spp. at the drainage point of the main stream represents the overall population of *Phytophthora* spp. within the upstream network. If our hypothesis is true, a stream network could be surveyed effectively at the drainage point without additional sampling of the upstream tributaries.

Two stream networks located in the Pisgah National Forest in western North Carolina were selected to test our hypothesis. The Davidson River stream network (watershed size of 35.2 km^2) is composed of the Davidson River and nine individual tributaries that drain nine sub-watersheds (fig. 1). Seven tributaries, each in a separate sub-watershed, and the drainage point at the lower end of the Davidson River were sampled in September and October 2007. The Cathey's Creek stream network (watershed size of 29.6 km^2) consists of a main stream, Cathey's Creek, and eight tributaries—each draining a sub-watershed (fig. 2). The drainage point of Cathey's Creek and eight tributaries were sampled in June and October 2008. The drainage points and tributaries in each stream network were sampled within a 30-minute time period to minimize potential temporal variation. Later, a second sample was collected at the drainage point when water from the tributary farthest upstream was estimated to reach this point based on a real-time stream flow model. A 1 liter water sample was collected at each sample site; nine 100-ml aliquots were filtered through polycarbonate membrane filters with 3-µm pores, and filters were inverted onto PARPH-V8 selective medium. After 72 hours, filters were removed, and colonies of *Phytophthora* spp. were counted. A bait bag with four

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detached *Rhododendron maximum* leaves was deployed at each drainage point to compare recovery of species of *Phytophthora* by filtration and leaf baiting. Bait leaves were retrieved 2 to 3 weeks after deployment and leaf disks taken from the edges of lesions were embedded in PARPH-V8 medium. Representative isolates were subcultured and identified based on morphological and molecular characters.

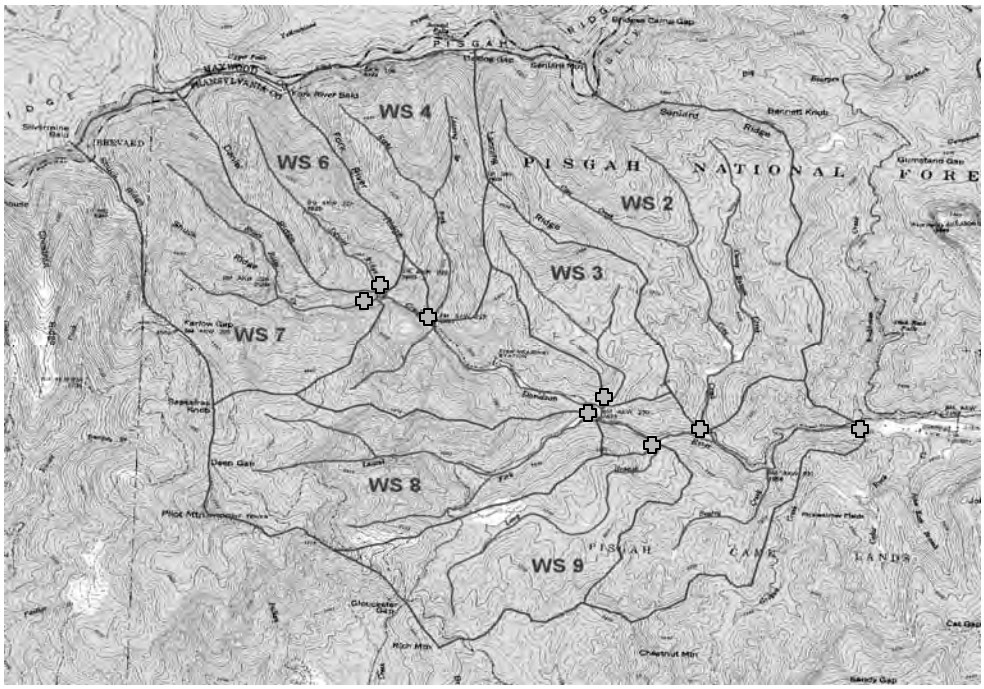

Figure 1—Sample sites (+), each in a distinct sub-watershed (WS), in the Davidson River stream network: the lower drainage point on Davidson River, Cove Creek (WS 2), Daniel Ridge Creek (WS 6), Laurel Fork (WS 8), Long Branch (WS 9), “No Name” Creek (WS 3), Right Fork (WS 4), and Shuck Ridge Creek (WS 7).

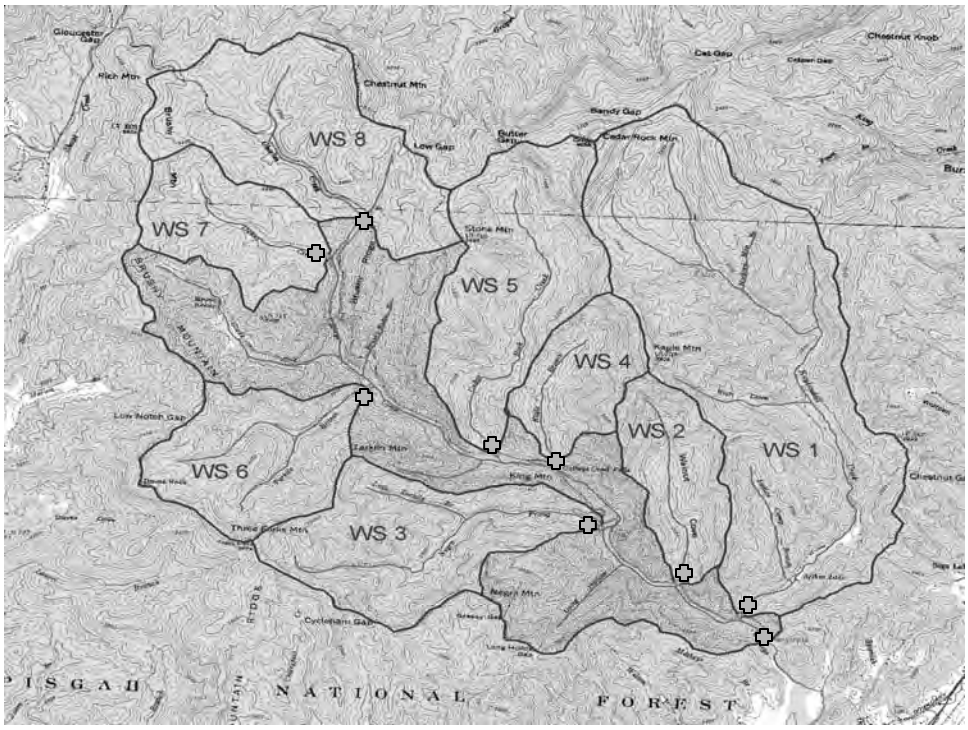

Figure 2—Sample sites (+), each in a distinct sub-watershed (WS), in the Cathey's Creek stream network: the lower drainage point on Cathey's Creek, Charles Creek (WS 8), Dunns Creek (WS 7), Tarkiln Branch (WS 6), Cedar Rock Creek (WS 5), Kagle Branch (WS 4), Negro Prong (WS 3), Walnut Cove (WS 2), and Kuykendall Creek (WS 1).

Six species of *Phytophthora—P. cinnamomi, P. citricola, P. citrophthora, P. gonapodyides, P. heveae,* and *P. pseudosyringae*—plus four other groups of isolates, which were morphologically and genetically distinct, were detected in the two stream networks. For each stream network, numbers of colonies and diversities of species varied among sample sites and between sample dates. Five species-groups were detected among 200 colonies recovered from the Davidson River network in September 2007, but only three of these were detected at the drainage point. In October 2007, nine species-groups were detected among 289 colonies recovered, and five species-groups were found at the drainage point. In the Cathey's Creek network, 155 and 219 colonies were recovered in June and October 2008, respectively, and seven species-groups were found at each sampling date. Three species-groups were detected at the drainage point in June, and five species-groups were found in October. The lower three tributaries in the Davidson River network had a higher mean density than that in the upper three tributaries (e.g., 41 vs. 10 colonies/900 ml, respectively). However, the upper three tributaries in the Cathey's Creek network had a higher mean density than that in the lower three tributaries (31 vs. 15 colonies/900 ml, respectively). With leaf baiting, three and two species-groups were detected at the drainage points on Davidson River and Cathey's Creek, respectively, during each sample period. All the species-groups found within a stream network were not detected at the drainage point. However, all of the species-groups present in the network that represented at least 9 percent of the total population were detected at this sample point. Based on this study, recovery of a species of *Phytophthora* at the drainage point is dependent upon the density of the population of that species in a forest stream network. Therefore, detection of a species present at a low population density may require more intensive sampling.

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Biosecurity Protocols for Heritage Gardens[1]

Ian Wright[2] and David Slawson[3]

Abstract

This project aims to protect The National Trust (NT) from the increasing number of harmful plant pests and diseases that slip though official controls and threaten our gardens, plant collections and landscapes.

During 2008, the National Trust (NT) with the seconded help of Dr. David Slawson, Head of Pest and Disease Identification Programme, Food and Environment Research Agency (FERA), United Kingdom (U.K.), has developed a suit of biosecurity protocols which we are now implementing at all of our 220 heritage gardens to help lessen the risk of introducing new pests and diseases to our properties. The NT, a U.K. charity formed over 100 years ago to "promote the permanent preservation for the benefit of the nation of sites of beauty and/or historic interest, forever for everyone," now is one of the largest garden owners in the world. Although the initial driver for the project was the impact of *Phytophthora ramorum* and *P. kernoviae* on the Trust's gardens, the lessons learned and the measures proposed will be of generic benefit across the increasing number of pests and pathogens that threaten the Trust's gardens and landscapes.

Biosecurity. 'Protecting Ourselves and Others'

During 2008, the National Trust (NT) with the seconded help of Dr. David Slawson, Head of Pest and Disease Identification Programme, Food and Environment Research Agency (FERA), has developed a suit of biosecurity protocols which we intend to implement at all of our 220 heritage gardens to help lessen the risk of introducing new pests and diseases to our properties.

The National Trust is a United Kingdom (U.K.) charity formed over 100 years ago to: "promote the permanent preservation for the benefit of the nation of sites of beauty and/or historic interest, forever for everyone." Now one of the largest garden owners in the world, it has an estimated 70,000 woody taxa in its gardens. The NT also manages 32 national plant collections and 28 internationally important collections.

The importance of the NT's collections to worldwide biodiversity has been recognized by, for example, being invited to join the Global Strategy for Plant Conservation as it relates to cultivated plants and plant collections. In addition to our concerns surrounding our important gardens we recognize the threat to our U.K. native species. Although not on NT land yet, the recent outbreaks in west Cornwall

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of *Phytophthora kernoviae* on *Vaccinium* (bilberry, an integral species of the native heathland habitat mosaic) has highlighted the urgency of addressing issues such as biosecurity to lessen any risk and impact to these important sites and further spread to other species.

It is vital that our gardens are managed in a way that creates an environment that is unfavourable for pests and diseases should they appear. The measures will help reduce the risk of gardens and plant collections becoming infested and the subsequent spread of pests and diseases from infested gardens via our visitors.

Project Aim - Preventative Conservation

The aim of the project is to protect the NT from the increasing number of harmful plant pests and diseases that slip though official controls and threaten our gardens, plant collections, and landscapes. Although the initial driver for the project was the impact of both *P. ramorum* and *P. kernoviae* on the Trust's gardens, the lessons learned and the measures proposed will be of generic benefit across the increasing number of pests and pathogens that threaten the Trust's gardens and landscapes. The output guidance should be simple, interesting, visual, relevant, user friendly, and cost conscious.

Biosecurity Project Outputs

Given these threats, it is vital that organizations such as NT, who are the custodians of some of the most significant gardens, parks, landscapes, and plant collections in Europe, take generic measures to protect these important assets. This project represents a start to that process. The project has undertaken a systematic review of both the plant pest and pathogen risks posed to the NT, and the adequacy of the NT's current procedures and practices.

The main output from the project is a series of Plant Quarantine and Biosecurity Guidance Notes, which aim to reduce the chances of new outbreaks occurring and minimize the damage should an outbreak occur. The guidelines include:
1. Sourcing plants.
2a. Handling brought-in plants and quarantine areas – general advice.
2b. Handling incoming plants and quarantine areas – for gardens of significant plant collections and the plant conservation programme.
3. Use of *Phytophthora* lateral flow devices (LFDs).
4. Cleaning footwear and hands.
5. Managing gardens to reduce the risk of pests and diseases.

A summary poster, containing guidance for visitors on how to help us to protect the garden from introduced pests and diseases, has been produced.

Guidance Note No. 5 is an as example of the depth of content. This note includes topics such as:

- plant husbandry (nutrition, spacing, pruning, plant debris, *Rhododendron ponticum*, hygiene);
- infrastructure (paths, fencing, signs);
- water (irrigation, drainage);

- waste disposal (burning, composting, deep burial); and
- monitoring (symptoms, awareness, contacts, training).

All elements provide examples of actual occurrences within normal garden management operations so that the users can relate closely to the written guidance.

Predictions and Cost/Benefit Analyses

It is virtually impossible to predict reliably the future development of outbreaks, and therefore, predicting accurately the cost/benefit analysis for adoption of the quarantine and biosecurity measures is equally difficult. Using *P. ramorum* as an example, we do know that it has killed millions of trees in coastal California, and is removing tanoaks (*Lithocarpus densiflorus*) from the tanoak – redwood (*Sequoia sempervirens*) climax vegetation ecosystem, the cost of which runs into millions of dollars. Closer to home, FERA predicts that, left unmanaged, *P. ramorum* and *P. kernoviae* will infest all susceptible gardens within 20 years. No estimate has been made on the spread to heathlands, but a similar prognosis is credible. A few reasonable scenarios comparing with and without enhanced biosecurity, are presented in table 1.

Table 1—Example scenarios comparing losses with and without enhanced biosecurity

Pest/pathogen	No enhanced biosecurity	Enhanced biosecurity
P. ramorum/ P. kernoviae	80 percent (160) of all TGs will be affected within 20 years (the remaining 20 percent will not be affected by virtue of the fact that they are on low-risk soil types). 100 percent of the Trust's collections of susceptible plant species will be affected within 20 years. Disease management costs of 160 outbreaks are estimated to be £5.5 million (based on the cost of the current outbreaks at Trust sites). Further costs will also be incurred for replanting the garden, lost revenue from reduced visitor numbers, and disease management of outbreaks on heathland sites.	8 percent (16) of all TGs will be affected within 20 years. 10 percent of the Trust's collections of susceptible plant species will be affected within 20 years. Disease management costs of 16 outbreaks are estimated to be £0.5 million (based on the cost of the current outbreaks at Trust sites). 90 percent reduction in costs will also be incurred for replanting the garden, lost revenue from reduced visitor numbers, and disease management of outbreaks on heathland sites.
Other pests and diseases	20 percent (40) of all Trust gardens will suffer a serious outbreak of a new plant pest or disease in the next 10 years.	2 percent (4) of all Trust gardens will suffer a serious outbreak of a new plant pest or disease in the next 10 years.
For example, Oak processionary moth	In the next 5 years, five gardens in London and the southeast will have to close at certain times because of public health concerns (asthma and urticaria rash).	In the next 5 years, one garden in London and the southeast will have to close at certain times because of public health concerns (asthma and urticaria rash).

Way Forward

The NT, recognizing the important need that we should all share responsibility and experience, has initiated the formation of a biosecurity working group made up of key stakeholders in the U.K. which will share good practice and guidance with other similar organizations, major garden owners, and the nursery trade. The objectives of the group are to:

1. Raise awareness amongst the general public.
2. Raise awareness amongst the professional gardening community.
3. Provide a single voice to government.
4. Create a fast communications network (email) group.
5. Explore ways in which valuable plants in U.K. collections can be protected for the very long term.

The NT will also look at introducing the following items to help raise awareness of staff, supporters, and general public by:

1. Including a quarantine and biosecurity module in the NT Careership (trainee gardener) programme.
2. Producing a guidance note for visitors (there are approximately 15 million visits per annum to NT gardens).
3. Commissioning an article for NT members magazine (issued to 3.5 million members).

Susceptibility of Australian Plant Species to *Phytophthora ramorum*[1]

Kylie Ireland,[2,3] Daniel Hüberli,[3,4] Bernard Dell,[3] Ian Smith,[5] David Rizzo,[6] and Giles Hardy[3]

Abstract

Phytophthora ramorum is an invasive plant pathogen causing considerable and widespread damage in nurseries, gardens, and natural woodland ecosystems of the United States and Europe, and is classified as a Category 1 pest in Australia. It is of particular interest to Australian plant biosecurity as, like *P. cinnamomi*; it has the potential to become a major economic and ecological threat in areas with susceptible hosts and conducive climates. Research was undertaken in California to assess pathogenicity of *P. ramorum* on Australian native plants. Sixty-eight test species within 24 families were sourced from established gardens and arboretums. Foliar and branch susceptibility were tested using detached leaf and branch assays. The experiment was repeated to account for seasonality. Initial results indicate the majority of species tested were susceptible to varying degrees. Of particular interest are the high levels of variability within the eucalypts, low levels of susceptibility within the Pittosporaceae family, and a concerning number of latent or asymptomatic infections.

Introduction

Phytophthora ramorum, the cause of sudden oak death in California, is an invasive plant pathogen causing considerable and widespread damage in nurseries, gardens, and natural woodland ecosystems of the United States (U.S.) and Europe (Werres and others 2001, Rizzo and others 2002, Brasier and others 2004). Classified as a Category 1 pest in Australia (Plant Health Australia 2006), it is of particular concern to Australian plant biosecurity as, like *P. cinnamomi*, it has the potential to become a major economic and ecological threat in areas with susceptible hosts and conducive climate.

Success of invasive organisms like *P. ramorum* in novel environments such as Australia relies on establishment in areas with conducive climates and susceptible hosts. The known worldwide host range of *P. ramorum* continues to grow, presently incorporating more than 100 species covering a diversity of trees, shrubs, and herbaceous species found in wildlands and nurseries (RAPRA 2007a, 2007b; USDA APHIS 2007). Two Australian host species, *Eucalyptus haemastoma* (scribbly gum) and *Pittosporum undulatum* (sweet pittosporum) have been listed as natural hosts of

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P. ramorum based on field observations and pathogenicity tests in the U.S. and Europe (Hüberli and others 2006, RAPRA 2007a, USDA APHIS 2007). In addition, *Leptospermum scoparium*, *E. gunnii* (cider gum) and *E. dalrympleana* (white mountain gum), have been found to be susceptible using artificial inoculation methods in the U.S., the United Kingdom (U.K.), and Spain respectively (Denman and others 2005, Huberli and others 2008, Moralejo and others 2009). A number of natural and associated host species of *P. ramorum* have been introduced to Australia. Douglas-fir, *Pseudotsuga menziesii*, for example, exists as a naturalized species (Lefoe 2002).

Given the wide and increasing host range of *P. ramorum*, including Australian native species, and evidence of a multiple-host method of dispersal (Moralejo and others 2006), it is expected that many Australian native plant species are susceptible. Research into the potential host range of Australian native species was undertaken in California to make an accurate assessment of the risk that it may pose to Australian plant biosecurity. Detached foliar and branch assays were used to assess the susceptibility of a range of key Australian native species to *P. ramorum*.

Methods

A total of 68 test species within 24 families were sourced from established gardens and arboretums in northern California. Species were selected based upon provenance from areas of climatic suitability for *P. ramorum*, as well as ecological and economical importance to Australian plant industries. Species were duplicated where possible from different locations or accessions. A total of 135 individual plants were tested. The known susceptible host *Rhododendron* 'Colonel Cohen' was used as a positive control across all experiments.

Detached leaf and branch assays were used to test for susceptibility, following the protocols of Denman and others (2005) and Hüberli and others (2008) respectively, and using isolate Pr510 from the culture collection of the Rizzo lab (University of California, Davis, Davis, California). Experiments were conducted in the summer of 2008 and winter of 2008/2009. Wounded foliar inoculations were only done during the summer. All plant material were kept in moist chambers at 20 to 25 °C and 16 hours daylight during summer, and 15 to 20 °C and 12 hours daylight during winter. The *Phytophthora* selective medium PARP was used for both foliar and branch studies to confirm infection.

Preliminary analysis of the results was undertaken to compare the species amongst one another, based only on averages of the recorded data. Foliar susceptibility was grouped based upon disease severity (confirmed by re-isolation) into non-hosts (0 percent), low susceptibility (<15 percent), moderate susceptibility (15 to 30 percent), high susceptibility (30 to 45 percent), and very high susceptibility (>45 percent). For branch susceptibility, species were ranked from smallest to largest mean lesion length.

Results and Discussion

Foliar inoculation results indicate that the majority of species tested are susceptible to *P. ramorum* in varying degrees. Only *Hedycarya angustifolia* tested completely

negative in both wounded and unwounded inoculation rounds. No trends were found to indicate particular plant families or genera to be more susceptible than another. Of note is that both *Isopogon* species tested were highly susceptible, and as they are important species in the international cut flower and nursery industries, could prove to be important in disease spread or epidemiology in Australia and abroad. *Agonis flexuosa* tested moderately to highly susceptible depending on their provenance. *A. flexuosa* is a common West Australian native planted widely as a street tree both on the east coast of Australia and West Coast of California, making it an ideal candidate for spread of the disease through native and nursery settings. Many eucalypt species tested highly susceptible, including *E. paucifolia* (snow gum), *E. sideroxylon* (red ironbark), and *E. nitens* (shining gum), an important native forest and plantation species.

Results of summer branch inoculations indicate less highly susceptible species than those with foliar inoculations. *Isopogon formosus*, *E. nitens*, and *E. sideroxylon*, all highly susceptible in foliar inoculations, were again highly susceptible on branches. Interestingly, *Hedycarya angustifolia*, not susceptible at all in the foliar trials, was highly susceptible in branch inoculation trials. In general, many of the eucalypts appear to be susceptible to *P. ramorum* branch dieback. No correlation between foliar and branch inoculation was detected.

Some basic trends have been observed so far. In both foliar and branch inoculations, higher susceptibility was observed in juvenile foliage and branches, while wounding increased susceptibility in foliar studies. The conifers and grasses tested were all low to moderate in susceptibility. Much variability was observed within species, genera and family levels, indicating *P. ramorum* is a generalist pathogen, and negating the possibility of predicting host range possibilities without testing a number of species, and individuals within those species. Some hosts, such as *E. leucoxylon*, *E. sideroxylon*, and *I. formosus*, appear to be both foliar and branch hosts of the pathogen, and as such, populations of these species may be at risk in the wild or in the nursery. Many species on the other hand, while being foliar hosts to some degree, were far less susceptible on their branches (*N. cunninghamii*, *O. argophylla*). On the other hand, *H. angustifolia* was highly susceptible in branch inoculations, with no observed foliar susceptibility. Initial results also indicate seasonal variability, as has been shown in previous inoculation studies with *P. ramorum* (De Dobbelaere and others 2007, Tjosvold and others 2007).

These results clearly indicate the potential for *P. ramorum* to infect and colonize a range of Australian native plant species from different families. Inoculum concentration, foliar sporulation, and log susceptibility will be investigated shortly. These results will provide the basis for climate and spread models to predict the pathogen's spread and impact on Australian plant industries and natural ecosystems should an incursion occur. Risk predictions generated by the models, and an understanding of the pathogen's host range, will allow the targeting of high-risk areas for early detection surveillance and protection, and assist regulators in developing appropriate quarantine protocols.

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Long-Term Trends in Coast Live Oak and Tanoak Stands Affected by *Phytophthora ramorum* Canker (Sudden Oak Death)[1]

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

Abstract

Permanent plots were established in 2000 to examine how tree and site factors affect risk of *Phytophthora ramorum* stem canker (sudden oak death [SOD]) and determine how affected stands change over time due to disease. *P. ramorum* canker was prevalent in the sampled coast live oak (*Quercus agrifolia*) or tanoak (*Lithocarpus densiflorus*) stands in 2000. In September of each year from 2000 through 2008, we collected data on *P. ramorum* symptoms, tree condition, tree failures, regeneration, and various other factors in 150 circular plots (8 m radius) at 12 locations in Marin, Sonoma, and Napa Counties.

Disease development patterns over time differed between tanoak and coast live oak populations in the study (fig. 1). The increase in disease incidence between 2000 and 2008 was greater for tanoaks (31 to 49 percent) than for coast live oak (23 to 33 percent). Disease incidence in coast live oaks increased strongly from 2005 through 2007. This increase was associated with abundant late season rains that provided favorable conditions for disease spread in spring 2005 and spring 2006. Tanoak showed a less much pronounced increase in disease incidence for these two years. Based on time lags between favorable conditions for infection and the appearance of visible symptoms, most coast live oaks had latent periods from 0.5 to 1 year, whereas the minimum latent period in tanoaks was commonly about a year.

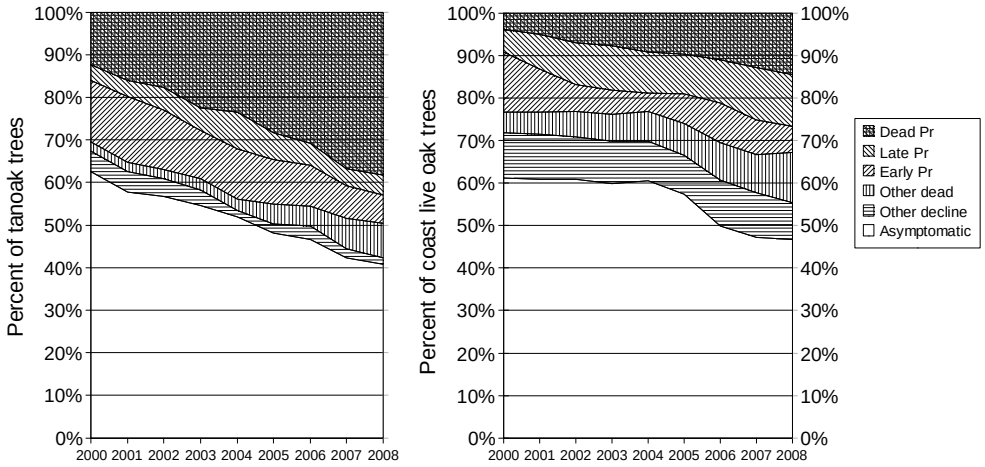

Figure 1—Changes in health of all tanoak (n = 187) and coast live oak (n = 655) study trees from September 2000 to September 2008. Dead Pr = tree dead as a result of *P. ramorum*; Late Pr = live trees with *P. ramorum* cankers plus beetle boring and/or *Hypoxylon thouarsianum* fruiting bodies; Early Pr = live trees with *P. ramorum*

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cankers only; Other dead = tree dead due to agents other than *P. ramorum*; Other decline = tree in severe decline due to agents other than *P. ramorum*; Asymptomatic = no evident symptoms of *P. ramorum* infection or decline due to other agents.

The increase in SOD mortality over time has followed a linear trend between 2000 and 2008 ($R^2 = 0.98$) for both oaks and tanoak. Extrapolating the regression lines back in time suggests that mortality would have initially appeared in the tanoak plots around 1996 and in the coast live oak plots around 1997. These estimated dates for onset of mortality are close to the first reported deaths of tanoaks in Marin County in April 1995 (Svihra 2001). Extrapolation of the regression line obtained for total tanoak infection ($R^2 = 0.96$) suggests that the first canker symptoms in these stands could have developed in the mid 1980s.

In 2008, disease incidence at coast live oak locations ranged from 8 to 57 percent. The increase in disease incidence since 2000 has varied widely by location. Relatively stable differences in disease incidence between nearby coast live oak locations were mainly associated with differences in California bay laurel (*Umbellularia californica*) cover rather than weather and climate variables. Disease risk of individual coast live oaks increased with increasing proximity and density of bay laurel foliage.

We also reexamined other factors previously associated with SOD risk in individual coast live oaks to account for changes in disease status due to new infections that developed in 2005 and 2006. Previously significant relationships were unchanged. Increased SOD risk was associated with greater bark thickness, which is positively correlated with stem diameter; higher amounts of active bark expansion, as expressed by unweathered bark in fissures; and lower than average water stress, as indicated by higher (less negative) stem water potential readings.

After the onset of symptoms, tanoaks showed high initial rates of mortality: 40 percent were killed within a year after the onset of symptoms and 63 percent were dead by 2008. In contrast, among coast live oaks first showing symptoms in 2001 or later, only 13 percent died within one year and total mortality had only reached 21 percent by 2008. Symptom remission, in which cankers stopped bleeding and showed no evidence of expansion for multiple years, was common in coast live oak. Among all trees observed since 2000, cankers became inactive in about 25 percent of the trees. In contrast, cankers became inactive in 46 percent of the trees that developed symptoms in the 2005 to 2006 disease pulse. Many of the cankers developing in 2005 or later were first observed while they were relatively small and may have been more likely to become inactive than larger cankers detected at a later stage. In addition, trees that were infected by *P. ramorum* in 2005 and 2006 represent trees that survived an earlier round of disease and could represent the more resistant trees in the population. Among all trees in which *P. ramorum* symptoms were verified by isolation of *P. ramorum* on selective media, 22 percent of the cankers became inactive.

The overwhelming majority of all host tree failures through 2008 (69 percent) occurred in trees that had *P. ramorum* canker symptoms in 2000. Early *P. ramorum* canker symptoms (bleeding only) were not associated with tree failure; only SOD-affected trees colonized by secondary organisms had elevated failure rates. Over two thirds of the initial failures in SOD-affected coast live oaks occurred in dead trees or dead stems of live trees. Relatively few trees that have become symptomatic since 2000 had failed by 2008. Trees rated as in decline or dead due to factors other than SOD also failed at elevated rates.

A survival analysis for all coast live oaks indicated that time to failure was most strongly affected by whether the tree was already dead (table 1). *P. ramorum* symptoms, *Hypoxylon thouarsianum* sporulation, and severe tree decline associated with other agents (typically decay fungi) also had highly significant effects on time to failure. Among live trees, time to failure was most strongly related to *H. thouarsianum.* Time to failure decreased as the proportion of the stem circumference with *H. thouarsianum* fruiting bodies present increased.

Table 1–Significance of parameters in a survival analysis (exponential model fit) for years to failure in coast live oak; predictor variables reflect conditions that existed in the year prior to failure

Source	DF	Prob>ChiSq
Overall model		<0.0001
Tree dead	1	<0.0001
P. ramorum symptoms	1	0.0025
H. thouarsianum sporulation	1	0.0030
Tree in decline	1	0.0077
Beetle boring	1	0.0414

Among plots with SOD-related mortality, 27 percent of the tanoak plots and 45 percent of the coast live oak plots showed decreases in plot canopy cover. Since 2001, coast live oak plots have shown an increase in Douglas-fir (*Pseudotsuga menziesii*) density (+31 percent), no change in California bay laurel density, and decreased madrone (*Arbutus menziesii*) density (−7 percent). Three locations had relatively high levels of madrone mortality, ranging from 17 to 31 percent. We have determined that *P. cinnamomi* is associated with declining madrone and bay laurel at one of these locations.

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Impacts of *Phytophthora ramorum* on Oaks and Tanoaks in Marin County, California Forests Since 2000[1]

Brice A. McPherson,[2] David L. Wood,[2] Maggi Kelly,[2] Sylvia R. Mori,[3] Pavel Svihra,[4] Andrew J. Storer,[5] and Richard B. Standiford[6]

Abstract

The forests of Marin County were among the first in coastal California to be affected by the *Phytophthora ramorum* epidemic. Although initially observed in 1994 in tanoaks (*Lithocarpus densiflorus*) and 1995 in coast live oaks (*Quercus agrifolia*), it is evident from studies of disease progression that the pathogen was present at least several years prior to the first recorded tree mortality (Rizzo and Garbelotto 2003; McPherson and others 2005). The causal agent had not been identified in March 2000 when we established 20 plots in two Marin County sites, China Camp State Park, (CCSP, coast live oaks and California black oaks, *Q. kelloggii*) and the Marin Municipal Water District watershed (MMWD, all three species). Plots were between 320 m^2 and 3600 m^2, with a mean of 1234 (SE = 199) m^2. The goal of the study was to monitor disease progression (McPherson and others 2000) and, in particular, to understand the phenomenon of the abundant beetle attacks observed on bleeding trees. Plots were evaluated four times per year until March 2003, then twice annually thereafter. Through March 2008, every stem > 5 cm diameter at breast height (DBH) was evaluated for signs and symptoms of sudden oak death (SOD): bleeding, beetle attacks, and the presence of the fungus *Hypoxylon thouarsianum*. In 2001 and 2007, we recorded the basal area for every woody species with stem DBH > 5 cm found within 0.08-ha subplots placed within the larger plots.

Mortality of each of the three species has increased steadily since 2000. Calculated infection and mortality rates were similar for coast live oaks and black oaks, but considerably greater for tanoaks (table 1). Mortality not attributable to *P. ramorum* serves as an estimate of background mortality. The proportion of the total living trees that were symptomatic, recorded at 1-year increments, increased gradually for coast live oaks and black oaks, whereas tanoaks exhibited a rapid increase from 2000 to a peak in 2004, then declined. This decline is likely a result of decreasing numbers of live stems in these heavily impacted sites.

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Table 1—Infection and mortality rates for coast live oak, black oak, and tanoak from 2000 to 2008 (percent y^{-1})

Species	Infection	Mortality	Non-*P. ramorum* Mortality
Coast live oak	5.0	3.1	0.54
Black oak	4.1	2.4	--
Tanoak	10.0	5.4	0.75

We modeled the decreasing numbers of asymptomatic coast live oaks and tanoaks resulting from the disease, using the over-dispersed Poisson regression. A regression line was fitted using the total number of asymptomatic trees from each plot with time as the independent variable, from March, 2000 to March, 2008, with plot nested in site as a random effect to account for the over-dispersion due to the repeated measurements. Asymptomatic coast live oaks were projected to decrease 50 percent by 2012 and 90 percent by 2036. For tanoaks, a 50 percent decrease occurred by 2005, with a 90 percent decrease projected by 2015.

Infection rates increased for all three species as a function of increasing stem DBH. Beetles have been shown to preferentially attack the larger bleeding coast live oaks (McPherson and others 2005). Numbers of attacks increased with increasing canker size (McPherson and others 2008). As a consequence, the mean size of surviving trees has decreased since 2000.

Disease stage-specific mortality was modeled using Cox proportional hazards (PH) analysis and Weibull survival analysis (Lee and Wang 2003). For each species, we modeled the overall survival of all trees that were asymptomatic in 2000. We then applied the analysis to initially asymptomatic cohorts of trees that 1) developed bleeding, and 2) developed bleeding and then were colonized by beetles. Variables included site (CCSP or MMWD), plot, and DBH. Each tree was assessed twice per year, in March and September, from 2000 to 2008.

Survival functions in both models were comparable, with the Weibull curves all lying within the 95 percent confidence intervals of the corresponding Cox PH curves. Median Weibull survival estimates (the point where the probability of surviving longer than a given time is 0.5) for trees that were asymptomatic in 2000 were similar for coast live oaks and black oaks, but less for tanoaks (table 2). For each species, estimates for trees that developed bleeding were 25 percent to 50 percent lower than the overall estimates. Beetle attacks further reduced median survival by 65 percent to 80 percent.

Table 2—Estimated median survival times (years) for initially asymptomatic trees (SE)

Species		Overall Asymptomatic	Bleeding	[1]Bleeding + Beetles
Coast live oak	CCSP	15.8 (1.5)	11.7 (2.7)	2.4 (0.6); 3.4 (0.8)
	MMWD	11.7 (0.8)	7.5 (1.6)	1.9 (0.5); 2.6 (0.5)
Black oak		13.8 (3.0)	6.2 (1.3)	1.9 (0.9)
Tanoak		8.8 (0.7)	5.9 (0.7)	1.7 (0.4)

[1]The two values shown for coast live oaks with bleeding and beetles represent cohorts with mean DBH = 20 cm (upper entry); mean DBH = 40 cm (lower entry).

The survival analyses reported here update earlier Weibull analyses that differed in two respects. In the earlier model, each same-symptom disease status cohort was composed of individuals with the same symptom status in March 2000, and trees were evaluated once per

year (McPherson and others 2005). By 2008, sufficient numbers of trees had progressed from asymptomatic to bleeding and ultimately to death that we could use only asymptomatic trees as the starting cohort. The value of the Weibull modeling approach is evident in the consistency of the estimates derived for symptomatic trees in both the 3- and 8-year time frames. Although the overall survival estimates for coast live oaks probably overestimated median survival times in the earlier model, 29.5 (SE = 8.4) y and 31.8 (9.3) y, survival estimates for bleeding trees were 7.0 (1.2) y and 7.6 (1.6) y, and for beetle-colonized trees, 2.6 (0.3) y and 3.2 (0.6) y, for CCSP and MMWD, respectively (McPherson and others 2005). For tanoaks, the earlier estimate for overall median survival was 12.6 (3.8) y, for bleeding trees, 8.7 (2.3) y, and for beetle-colonized trees, 2.9 (1.0) y. In general, the modeled median survival estimates for symptomatic coast live oaks and tanoaks were more consistent for the two models than were the estimates for overall survival. Once beetles attacked trees, both models showed dramatic, consistent decreases in survival.

Basal area of all woody stems was measured in 2001 and 2007 in 0.08 ha plots sited within the larger plots. Live California bay laurel (*Umbellularia californica*) basal area increased by 8 percent during this period. The proportion of coast live oaks in the disease progression plots that were symptomatic and dead was positively related to bay laurel stand basal area in 2001 ($F_{1, 14} = 13.096$, $P < 0.0028$) and in 2007 ($F_{1, 14} = 4.99$, $P < 0.042$). The diminished strength of the relationship suggests that the role of bay laurel in *P. ramorum* transmission to coast live oaks was more important early in the epidemic and decreased with time. Live Pacific madrone (*Arbutus menziesii*) basal area decreased 12 percent during the same period. There was a negative relationship between madrone basal area and the proportion of coast live oaks that were symptomatic and dead. The strength of this negative relationship increased from 2001 ($F_{1, 14} = 3.41$, $P > 0.086$) to 2007 ($F_{1, 14} = 5.16$, $P < 0.0028$). We also found an inverse relationship between basal area of bay laurel and madrone in these plots. We hypothesize that the contrasting trends of the relationship between basal area of bay laurel and madrone is correlated with moisture availability or with variables dependent on moisture availability, such as the infection process. In these forests Pacific madrone tends to be found in drier sites and bay laurel is more dominant in wetter sites.

This study of the responses of individual host trees supports wider use of survival analysis to model ecological change driven by introduced pathogens. Because relatively few variables are required to develop predictions of change at the stand and landscape scales, survival analysis can serve as a useful tool for land management decisions.

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Sudden Oak Death in Redwood Forests: Vegetation Dynamics in the Wake of Tanoak Decline[1]

Benjamin Ramage[2] and Kevin O'Hara[2]

Introduction

Numerous lines of inquiry have concluded that tanoak (*Lithocarpus densiflorus*) will continue to experience drastic population declines and may even disappear entirely from redwood (*Sequoia sempervirens*) forests as a result of the exotic disease sudden oak death (SOD) (Maloney and others 2005, McPherson and others 2005, Meentemeyer and others 2004, Rizzo and others 2005). As the only species that can both compete for canopy space and tolerate the deep shade of redwood understories, tanoak is widespread and abundant in redwood forests, and is an integral component of the structure and function of these unique ecosystems. Tanoak is the most common hardwood species in conifer forests of California's coastal mountains, and is associated with redwood throughout the majority of the redwood range (Burns and Honkala 1990, Hunter and others 1999, Noss 2000). As such, the loss of tanoak from redwood forests is likely to result in significant impacts. The primary objectives of this study were to: (1) examine the short-term compositional and regenerative effects of SOD in redwood forests; (2) determine which compositional and regenerative variables are correlated with tanoak abundance; and (3) consider the long-term structural and compositional effects of SOD-induced tanoak decline in redwood forests.

Materials and Methods

In order to assess the effects of SOD-induced tanoak decline, measurements of forest structure, shrub and herb cover and composition, and regeneration were collected in 65 plots distributed throughout three infested counties. Most plots were randomly located within second-growth redwood forest, but some supplemental plots were subjectively installed in order to ensure adequate representation of the full range of tanoak abundance and disease severity. All field research was conducted in a single field season (summer 2008), and thus we do not have time series data, but we nonetheless believe it is possible to reliably infer short-term impacts of SOD-induced tanoak mortality. Given that the current highly patchy distribution of SOD in redwood forests is believed to be a result of introduction events and stochasticity (Maloney and others 2005, Moritz and others 2008, Rizzo and others 2005), as opposed to confounding biotic or abiotic variables, it is likely that differences correlated with SOD are caused by SOD. In contrast, relationships between total

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tanoak abundance and other investigated variables could be controlled by other unmeasured factors (for example, soil nutrients), and thus we are hesitant to claim a causative role for tanoak abundance.

Field sites were located in the counties of Santa Cruz (The Forest of Nisene Marks and Henry Cowell Redwoods State Park), Marin (Marin Municipal Watershed District), and Humboldt (Humboldt Redwoods State Park). Data collection consisted of the following: (1) species, health status, diameter, and presence or absence of basal sprouts for all trees greater than 10 cm diameter at breast height; (2) cover and composition of shrubs and herbs; (3) tree regeneration tallies; (4) canopy cover and coarse woody debris; and (5) standard plot measurements such as slope, aspect, and elevation. Foliar samples were also collected in each plot to test for the presence of *Phytophthora ramorum*, the causative pathogen. Samples were processed by the University of California (UC) Berkeley forest pathology laboratory.

Data analysis consisted primarily of multivariate generalized linear modeling. This flexible technique allowed us to examine response variables with highly non-normal error distributions (for example, regeneration counts), and to isolate the effects of tanoak mortality and abundance while accounting for other uncontrolled variables. For all response variables, we specified the appropriate error distribution and started with the following full model: Y ~ dead tanoak abundance + total tanoak abundance + redwood abundance + slope + slope position + northness + Humboldt + Santa Cruz [Marin = baseline]. We then fit models with all possible subsets of predictors, and selected the “best” model (the model with the lowest AIC [Akaike's Information Criterion] value) for interpretation. In addition, we calculated simple averages of tanoak regeneration and mature tree abundance; for these calculations, we utilized randomly located plots only.

Results and Discussion

Our attempt to verify the presence of *P. ramorum* in diseased plots was unsuccessful. We were unable to verify the presence of *P. ramorum* in many plots with high mortality levels, but there are compelling reasons to suspect that these results represent false negatives: (a) our sampling period followed 2 very dry years, and dry conditions are known to decrease the likelihood of detection (Rizzo and others 2005); (b) two plots which tested negative were confirmed positive in a previous study that was conducted in a wetter year (Spencer 2004); (c) several plots that tested negative exhibited very severe levels of tanoak mortality and displayed other characteristic symptoms of SOD as well (in all of these cases, infestation has been confirmed in the general area); and (d) no other agent is known to cause such severe and widespread mortality of tanoak (Swiecki and Bernhardt 2006). In fact, because the four most severely impacted plots all returned negative results, we suggest that greater light penetration (and presumably lower humidity levels) may actually make it more difficult to detect the pathogen in severely diseased areas, at least in dry years. These results also suggest that the dry sampling year may have produced false negatives in plots with lower levels of mortality. As such, we consider these results unreliable and assert that most tanoak mortality has probably resulted from SOD. It is also worth noting that we are unable to prove every dead tanoak we encountered was killed by SOD; in fact it is highly likely that some individuals died of other causes (for example, competition, windthrow).

Impacts that we loosely attribute to SOD are actually, in a strict sense, impacts of tanoak mortality. However, since severe tanoak mortality is not associated with any disturbance other than SOD, and all of the effects that we have detected are most apparent at very high levels of mortality, we believe it is reasonable to assign a causative role to SOD.

Our results demonstrate the great abundance of tanoak in redwood forests within our study area, and also identify several variables that are related to SOD-induced tanoak mortality and/or total tanoak abundance. In all three counties, mean tanoak basal area, stem counts, and importance values were higher than all other hardwoods combined, as well as all conifers excluding redwood. In terms of regeneration, median tanoak regeneration exceeded all other species combined, in all three counties, and tanoak regeneration occurred in 100 percent of our randomly located plots; as such, tanoak is currently a ubiquitous component of the regeneration stratum in redwood forests.

After accounting for the effects of all other potential model predictors (total tanoak abundance, redwood abundance, slope, slope position, northness, and sampling site), SOD-induced tanoak mortality was positively associated with coarse woody debris, herbaceous species richness, the percent of total mature stems with basal sprouts, and the percent of mature tanoak stems with basal sprouts. SOD–induced tanoak mortality was negatively associated with total canopy cover. In addition, weak positive relationships (significance levels between 0.05 and 0.1) were found with regard to the percent of mature redwood stems with basal sprouts and the percent of mature hardwood (excluding tanoak) stems with basal sprouts. We discovered no effects of SOD-induced tanoak mortality on herbaceous cover, shrub cover, shrub richness, mature tree richness, or juvenile tree richness.

Notably, we did not detect a regenerative response to SOD-induced tanoak mortality (in terms of absolute counts, as opposed to the percent of mature stems with basal sprouts) for any regeneration category (total, tanoak, redwood, hardwoods excluding tanoak, conifers excluding redwood, and all species excluding tanoak and redwood). This result was surprising given the frequently observed – and often dramatic – deterioration of the canopy, a process that had already begun 7 years prior to field sampling in at least some of our plots (as documented by Spencer 2004). It is also worth noting that the plots with historical records were not as severely impacted as many other plots, and thus it is likely that some plots had been experiencing increased light levels for well over 7 years.

After accounting for the effects of all other potential model predictors (tanoak mortality, redwood abundance, slope, slope position, northness, and sampling site), total tanoak abundance was negatively associated with herbaceous cover, herbaceous richness, shrub richness, mature tree richness, the percent of mature redwood stems with basal sprouts, and the percent of mature non-redwood non-tanoak stems with basal sprouts. Total tanoak abundance was positively associated with tanoak regeneration, suggesting a tendency towards self-replacement in the absence of SOD.

In summary, our results suggest that as diseased tanoaks die and deteriorate, the forest canopy opens, coarse woody debris levels increase, and herbaceous species experience increased seedling recruitment and/or vegetative expansion. In addition, basal sprout incidence may weakly increase for some tree species, but a pronounced

and definitive regenerative response is unlikely to occur, at least in the first several years after mortality. Given the current paucity of regeneration in areas with high mortality, we have little evidence with which to predict what tree species will eventually occupy SOD-induced tanoak mortality gaps. Our data also illuminate correlations between total tanoak abundance and several variables of interest, but such relationships may be controlled by other variables that were not measured as part of this research. Long-term impacts of SOD-induced tanoak decline will depend upon the extent to which tanoak abundance controls other key variables, and how such effects will interact with short-term mortality patterns, tree regeneration, and resulting ecological trajectories.

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Community and Individual Effects on SOD Intensification in California Redwood Forests: Implications for Tanoak Persistence[1]

Richard C. Cobb,[2,3] Joao A. N. Filipe,[4] Ross K. Meentemeyer,[5] Chris A. Gilligan,[4] Shannon C. Lynch,[3] and David M. Rizzo[3]

Abstract

Processes operating across different spatial scales (for example, individual, community, landscape) influence disease dynamics. Understanding these processes and their interactions can yield general insights into disease control, disease dynamics within communities, and community response to disease. For *Phytophthora ramorum*, pathogen establishment and disease intensity are key drivers of deleterious impacts on ecosystems such as changes in fuel loads, tree mortality, and transformation of native plant communities. We studied infection rates of *P. ramorum* for major overstory species together with mortality rates caused by sudden oak death in tanoak (*Lithocarpus densiflorus*) in central coast redwood forests of California. Our analyses used a combination of individual and population-level estimates for these processes. Approximately 5800 trees were surveyed in 2002 and 2007 across 120 plots located at 14 sites from Sonoma to Monterey Counties (Maloney and others 2005). Our objectives were to examine rates of pathogen establishment amongst species and to identify key drivers of tanoak mortality. We used hierarchical path analysis to quantify community disease drivers (overstory species densities, prevalence of *P. ramorum* in infectious hosts) and survival analysis to examine characteristics that influence infection and mortality rates at the individual level (tree size, canopy position, species, post mortality sprouting). A simple theoretical model was constructed to examine the potential for tanoak persistence across redwood communities where *P. ramorum* has been naturalized. The model was parameterized from the path and survival analyses.

Previous study has shown the importance of the presence of California bay laurel (*Umbellularia californica*) and tanoak as factors predicting the likelihood of infestation (Maloney and others 2005). We hypothesized that disease severity at the stand level would be driven by the prevalence of infection within these two species. Furthermore, we expected that canopy position and tree size would cause infection and mortality rates to differ among tanoak trees. Our path analysis identified prevalence of infected tanoak and bay laurel as key drivers of tanoak mortality rate across our network of study plots. Pathogen prevalence in both species increased mortality rate, but the per-tree effect of bay laurel was 1.4 times greater than that of tanoak (Cobb and others, in press). High sporulation rate in bay laurel (potentially 10 times higher than tanoak; Davidson and others 2008) is the most likely driver of differences in mortality rate amongst our study plots, and suggests that risks posed by *P. ramorum* are best quantified among species transmission rates as opposed to among species susceptibility (Davidson and others 2008). Average time to infection differed among species. Within stands

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where *P. ramorum* had already become established, average time to infection was 8, 3, and 2 years for coast redwood (*Sequoia sempervirens*), tanoak, and bay laurel respectively. Considerable variation in time to infection was observed across plots and decreased with higher prevalence of infected bay laurel and tanoak, landscape level force of infection, and across canopy strata. Infection was more rapid in understory trees compared with trees in higher canopy strata. In contrast to rates of infection, median tanoak mortality rates following confirmed infection were more rapid in large trees compared with small trees. Once infection was confirmed in our study tanoak trees, trees died on average within 1 to 6 years, depending on tree size and number of infected bay laurel and tanoak within plots. Infection latency could bias these estimates towards an underestimate of post infection survival time. Survival times for all tanoak (regardless of confirmed infection status) resulted in death of tanoak on average between 4 to 15 years, depending upon other factors within plots. These estimates correspond closely with the time to infection following exposure (pathogen establishment within the stand) and post infection mortality. Estimated tanoak survival times were greatest for tanoak in stands dominated by conifers otherwise isolated from other infected bay laurel and tanoak. Within our study plots, trees with maximum expected survival were located in stands dominated by redwood and Douglas-fir (*Pseudotsuga menziesii*). Half of all tanoak biomass has been killed within our study plots during the first 8 years following disease establishment. However, 2.3 percent of the study population had estimated survival times greater than 28 years. This suggests potential exists for tanoak persistence at low densities and in stand conditions with low or no density of other species that support sporulation of *P. ramorum*, such as bay laurel and *Rhododendron* sp. Results from these statistical models are in line with results from our theoretical model (see below).

Stump sprouting from trees that have been killed by *P. ramorum* is common (43 percent of *P. ramorum*-killed tanoak stems) and 58 percent of sprouting basal sprouts had symptoms of *P. ramorum* infection. This suggests that of killed trees, 25 percent still provide an opportunity for vertical transmission via sprouting. Prolific basal sprouting also provides an additional pathway for pathogen persistence, especially in stands where tanoak is the sole supporter of sporulation. Post mortality basal sprouting may also help tanoak persist as an understory component of coast redwood forests. To test this hypothesis, we constructed a simple dynamic disease-competition model to investigate tanoak persistence with and without a co-occurring community member which supports sporulation. In our model, the co-occurring sporulation supporting species is similar to bay laurel in that infection does not alter the host mortality rate. The model suggests that in pure tanoak stands, tanoak persistence is likely, but surviving trees may not survive beyond 15 years which would relegate the species to the understory. In stands with sporulation supporting species such as bay laurel, tanoak is likely to persist only when post-mortality sprouting is high, but tanoak densities are expected to be much lower than in pure stands and tanoak would be relegated to the understory. This study demonstrates that sudden oak death differs from historical disturbances such as fire by selectively killing trees that are large and close to *P. ramorum* sporulating species. The disease is also likely to increase understory tanoak for the next several decades, but it remains uncertain whether these stand changes could impede regeneration of economically valuable conifer species. Forestry practices which increase conifer regeneration, such as thinning of tanoak and other sporulation supporting species, may also reduce the prevalence of sudden oak death and maintain tanoak as a component of coast redwood forest biodiversity.

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Management/Treatment

Efficacy of Commercial Algaecides to Manage Species of *Phytophthora* in Suburban Waterways[1]

G. Curtis Colburn[2] and Steven N. Jeffers[2]

Abstract

Many commercial algaecides contain copper compounds as active ingredients. *Phytophthora* spp. and other oomycetes are known to be sensitive to copper-based fungicides. Therefore, algaecides registered to manage algae in natural waterways and irrigation waters also might be effective for mitigating or even eradicating *Phytophthora* species, including *P. ramorum*, in these same waterways. Many of the commercially available algaecides are registered for use in diverse natural and agricultural water environments, and water treated with these products may be used for swimming, fishing, watering livestock, and irrigating turf and ornamental plants immediately after treatment. Consequently, these algaecides appear to be relatively safe for both humans and the environment. Experiments in our laboratory have demonstrated that two algaecides with copper-based active ingredients were toxic to zoospores, sporangia, and chlamydospores of *P. ramorum* and to zoospores of six other species of *Phytophthora*.

Chlamydospores (5 x 10^3 spores/ml), sporangia (2.5 x 10^3 sporangia/ml), and zoospores (1 x 10^5 spores/ml) of A1 and A2 isolates of *P. ramorum* were exposed to commercial rates of two algaecides (0.8 ppm of copper carbonate and 1.0 ppm of copper-triethanolamine + copper hydroxide) for 0, 0.5, 2, 4, 8, and 24 hours. Treated propagules were collected on membrane filters and then washed to remove algaecide residues. Filters were inverted on PAR-V8 selective medium, and plates were placed at 20 °C so propagules on the filters could germinate and produce colonies. For both isolates, zoospores were not recovered after 30 minutes of exposure to either algaecide. Compared to the non-treated control, viabilities of chlamydospores and sporangia of both isolates were reduced significantly at 2 and 4 hours of exposure to the algaecides; no chlamydospores or sporangia remained viable at 8 or 24 hours of exposure. In addition, zoospores of *P. cryptogea*, *P. nicotianae*, *P. palmivora*, *P. citricola*, *P. cactorum*, and *P. citrophthora* were exposed to each algaecide for 30 and 60 minutes. Zoospores from any of the species were not recovered at either 30 or 60 minutes of exposure to the algaecides.

To evaluate the efficacy of commercial algaecides to manage species of *Phytophthora* that occur naturally in suburban waterways, six streams in three urban communities in the northwestern region of South Carolina were selected, and water in each stream was collected and treated twice. These streams were known to be infested with naturally occurring populations of species of *Phytophthora* based on previous studies conducted by our research team. At each stream, each of twelve 20 liter buckets was filled with 15 liters of water in 1 liter aliquots. The buckets were returned to the laboratory and were held at room temperature (22 to 25 °C) for the duration of the experiment. Four buckets were not treated and served as controls, four buckets were treated with 0.8 ppm copper carbonate (Captain®, SePRO Corp.),

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and four buckets were treated with 1.0 ppm copper-triethanolamine and copper hydroxide (K-Tea®, SePRO Corp.). The rates used were those recommended on current product labels. Prior to treatment (0 hours), the initial mean density of propagules (as colony-forming units [cfu]/liter) was calculated for each set of four buckets. Density was determined by removing three 200 ml aliquots from each bucket and passing each aliquot through a polycarbonate membrane filter with 3 μm pores. Filters were inverted onto PARPH-V8 selective medium, and plates were held at 20 °C for 2 to 3 days. Filters then were removed and colonies of *Phytophthora* spp. were counted. After algaecides were added, the water in each bucket was stirred periodically and sampled at 1 and 4 hours to determine density of *Phytophthora* spp. Filters receiving aliquots from treated water were washed with distilled water to remove algaecide residue before being placed on selective medium. Mean density in each treatment was calculated for each sample time for each of the six streams, and data were analyzed independently for each stream.

Results from the two trials for each stream were similar, so data were combined for analysis. Initial densities of *Phytophthora* spp. in the six streams varied and ranged from 38 to 136 cfu/liter. For each stream, initial densities for the three treatments were not significantly different ($P = 0.05$). In the non-treated control from each stream, densities at 1 and 4 hours did not change significantly from that at 0 hours. However, *Phytophthora* spp. were not recovered in water from any stream that had been treated with algaecide; in other words, no colonies developed on isolation plates after 1 or 4 hours of exposure to algaecide. In all six streams, densities of *Phytophthora* spp. in treated water at 1 and 4 hours were significantly different from densities at 0 hours and from densities in non-treated water. In summary, commercial algaecides used at rates recommended on product labels appear to have excellent potential to manage *Phytophthora* spp. in waterways.

Acknowledgments

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Aerial Application of Agri-Fos® to Prevent Sudden Oak Death in Oregon Tanoak Forests[1]

Alan Kanaskie,[2] Everett Hansen,[3] Wendy Sutton,[3] Paul Reeser,[3] and Carolyn Choquette[3]

Abstract

We have been testing the practicality and efficacy of aerial application of Agri-Fos® for control of sudden oak death (SOD) in the Oregon tanoak (*Lithocarpus densiflorus*) forest. Helicopter application to forest stands has been compared with bole injection and ground-based spray application to seedlings and stump sprouts. We bio-assayed for Agri-Fos® uptake using twig and seedling inoculation with zoospores and bole wound inoculation. Experiments are continuing. Preliminary results indicate small but significant reductions in growth of *Phytophthora ramorum* in Agri-Fos® sprayed trees, comparable to the results obtained from bole injection.

Introduction

Phosphonate (Agri-Fos®) has been shown to be effective in limiting development of *Phytophthora ramorum* when sprayed directly onto or injected into individual tree boles. There have been no tests, however, of its application and uptake under forest conditions, or of its efficacy as a disease protectant in landscape scale application on tanoak (*Lithocarpus densiflorus*). Proper governmental registration and permission for use of phosphonate in the forest requires demonstration of efficacy. We are conducting trials in Oregon to demonstrate the feasibility of aerial application from helicopter, phytotoxicity at various spray concentrations, and the uptake and translocation of the chemical within treated trees. We are also testing the efficacy of Agri-Fos® as a protectant fungicide by spraying tanoak seedlings and then exposing them to natural inoculum of *P. ramorum* in the field. Results presented here are preliminary and do not represent the complete data set. Final results and analysis will follow completion of the final assays in November 2009.

Methods

Three methods of Agri-Fos® application are being compared: helicopter application to mature tanoak forest trees; backpack pressurized spray to simulate the helicopter application to tanoak seedlings and sprouts; and bole injection of mature tanoak trees. The aerial spray application rates were: no treatment; 3 gal Agri-Fos® 400/20 gal water/acre; and 6 gal Agri-Fos® 400/20 gal water/acre. The seedling application rates were the same as the aerial rates except the 6 gal per acre rate was replaced by 0.1 gal

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Agri-Fos® 400/20 gal water/acre. Pentra-Bark® was added to all spray mixes. Tanoak trees were injected with Agri-Fos® 400 at the label rate of 10 ml injection fluid (1 part Agri-Fos®:2 parts water) per 15 cm trunk circumference.

Five different biological assays are being used to measure Agri-Fos® uptake:
1. Twig assay: Ten treated trees per treatment plot were felled and 20, 25 cm long shoot tips (twigs) from the outer canopy were collected from each and transported to the laboratory. Cut ends of shoot tips from forest trees or sprout clumps are immersed in a 1 cm deep zoospore suspension then incubated 3 weeks. Resultant lesion lengths are measured.
2. *In situ* bole inoculation with *P. gonapodyides*: Intact tanoak trees are wound inoculated in the field. Lesion area is measured after 5 weeks. *P. gonapodyides* was used in field inoculations instead of *P. ramorum* due to quarantine regulations.
3. "Log" bolt inoculation with *P. ramorum*: Trees are felled and 1 m bolts cut and returned to the laboratory. Logs are wound inoculated in the laboratory, and lesion area measured after 5 weeks.
4. Artificial inoculation seedling assay: Treated seedlings are challenge inoculated with a zoospore and sporangium suspension of *P. ramorum*, and incubated in a growth chamber until symptoms develop.
5. Treated seedlings are exposed to natural inoculum beneath infected tanoak trees in Curry County, Oregon.

Treatment areas for helicopter spray treatments were selected in extensive stands of tanoak north of the SOD infestation in Curry County, Oregon. Treatments were applied by contract helicopter November 2007, May 2008, December 2008, and May 2009 (table 1). At each application time, we sprayed three blocks, each consisting of three 10 acre treatment plots.

Table 1—Aerial application of Agri-Fos® to tanoak forests: tests, treatments, and assays

Test	Spray Dates	Treatments	Area Treated	Assay	Assay Date
1	Nov 07	0, 3, and 6 gal /Acre	3, 10 Acre Blocks/Treatment	Branch dip In situ *P. g*	May 08
2	May 08	0, 3, and 6 gal /Acre	3, 10 Acre Blocks/Treatment	Log-*P.ram* Branch dip In situ *P. g*	Jan 09 Nov 09
3	Nov 08 and May 09	0, 3, and 6 gal /Acre at each time	3, 10 Acre Blocks/Treatment	Branch dip In situ *P. g*	June 09 Nov 09

Results

Aerial Application

The twig assay (fig. 1), the *in situ* assay with *P. gonapodyides* (fig. 2), and the log assay with *P. ramorum* (fig. 3), all indicated an Agri-Fos® effect in sprayed trees 6 months or 18 months after treatment. Lesion length differences with the twig assay were usually small, but significant. The *in situ* and log assays gave larger and more consistent differences in lesion area between treatments. There was no consistent difference between the two fungicide doses.

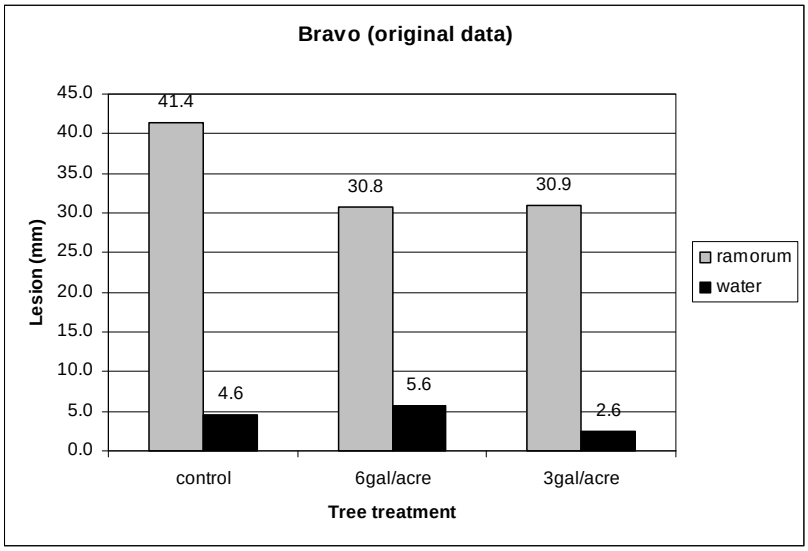

Figure 1—Aerial application of Agri-Fos®: twig assay. Data from one plot in the fall 2008 application.

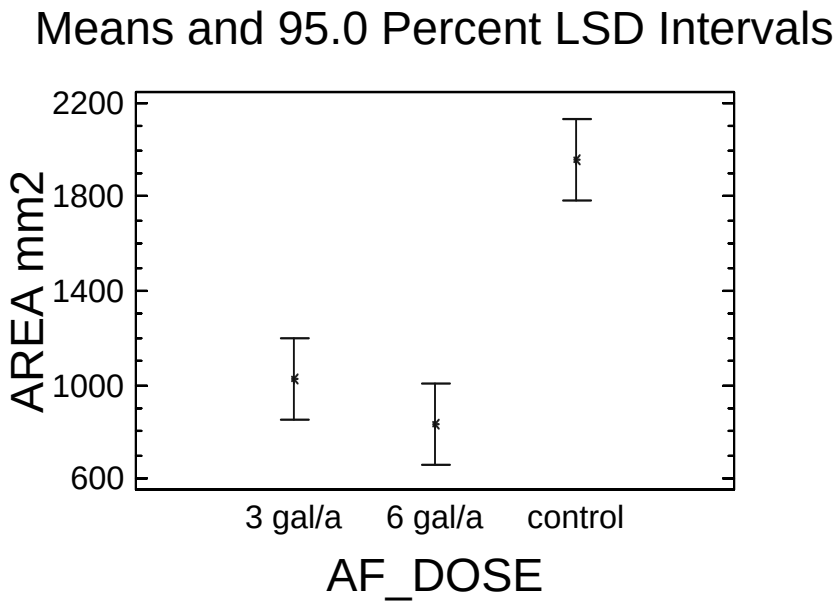

Figure 2—Aerial application of Agri-Fos®: *in situ* bole inoculation assay with *P. gonapodyides.* Average lesion area for all sites after November 2007 application.

Results from double application of Agri-Fos® (treatment three, sprayed November 2008 and May 2009) will not be available until final assays are completed fall of 2009. At that time the overall project will be evaluated and results reported.

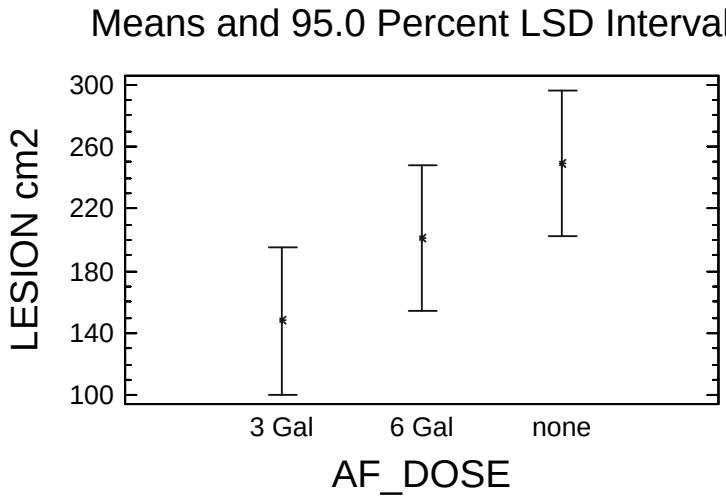

Figure 3—Aerial application of Agri-Fos® (AF): log inoculation assay with *P. ramorum*. Lesion area data from all sites after the May 2008 application.

Bole Injection

Ten Agri-Fos® injected trees were paired with control trees in both 2007 and 2008 trials. The shoot dip assay showed slightly smaller lesion length in the injected trees compared to controls. Bole inoculation with *P. gonapodyides* as well as log inoculation with *P. ramorum* showed similar but larger differences between injected and control trees (fig. 4 and 5; table 2).

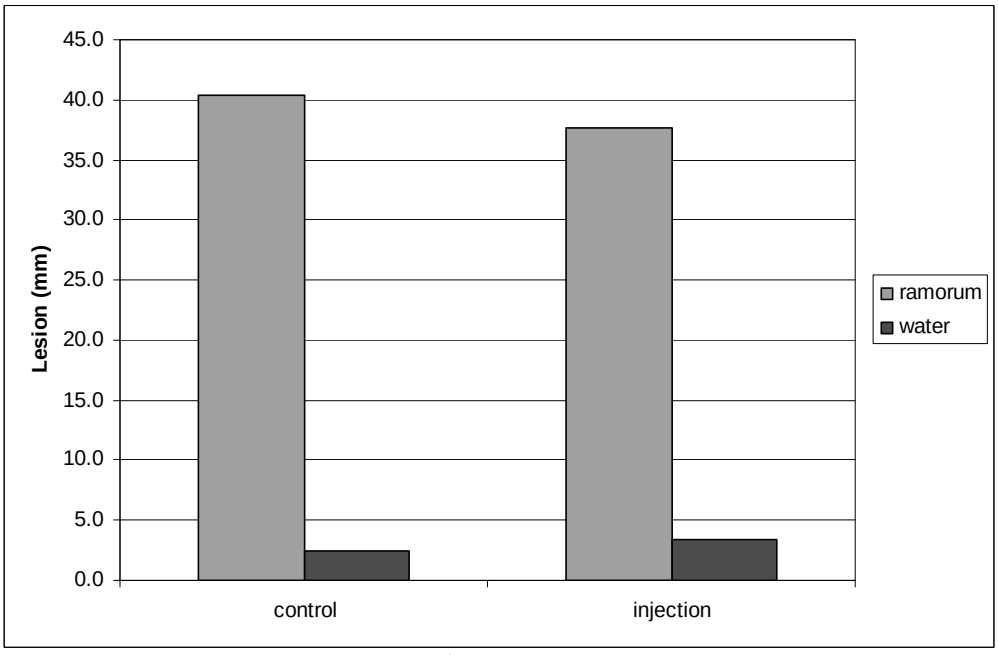

Figure 4—Tree injection with Agri-Fos®: twig assay from the 2007 trial.

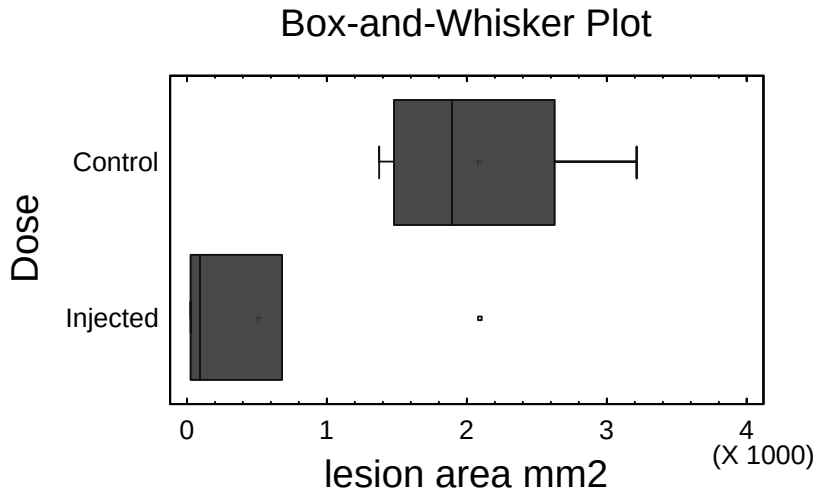

Figure 5—Tree injection with Agri-Fos®: *in situ* bole inoculation assay with *P. gonapodyides*. 2008 trial.

Table 2—Tanoak bole injection with Agri-Fos®: summary of tests and assays

Assay	Injection Date			
	NOV 2007		MAY 2008	
	Agri-Fos®	Control	Agri-Fos®	Control
Twig (mm)	37.6	40.4	45.8	47.6
Bole Pg (cm^2)	5.0	20.8	0.4	24
Log Pg (cm^2)	x	x	29	63
Log Pr (cm^2)	x	x	82	206

Stump-Sprout Foliar Spray Treatments

There were no obvious symptoms of phytotoxicity resulting from the spray treatments. There were significant differences ($p = 0.00$) in sprout response to challenge inoculation; lesion length on sprouts sprayed at the high dose (6 gal/acre) averaged 44 mm, and control, unsprayed sprouts averaged 78 mm.

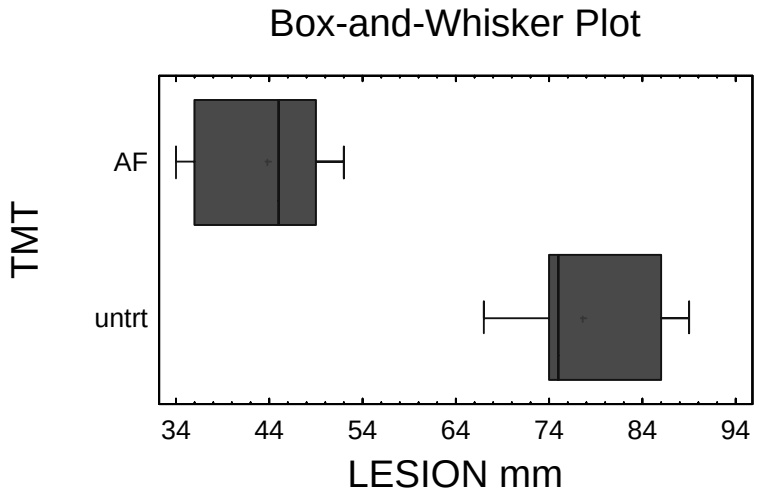

Figure 6—Stump sprout spray with Agri-Fos® (AF) compared to untreated control (untrt): twig assay. Data from 2008 trial.

Seedling Spray Treatments

Seedlings were sprayed in October 2008 and in April 2009, with one set of seedlings sprayed in both October and again in April, then challenge-inoculated with *P. ramorum* in the lab or exposed to natural inoculum in the field. In October, only incidence of infection was recorded. In April, both the percent seedlings infected and the extent of stem infection (proportion of total stem length) that was infected were recorded (fig. 7 and 8). Agri-Fos® did not prevent infection by *P. ramorum* in any treatment, and the effect of Agri-Fos® dose on incidence and extent of infection was variable between trials.

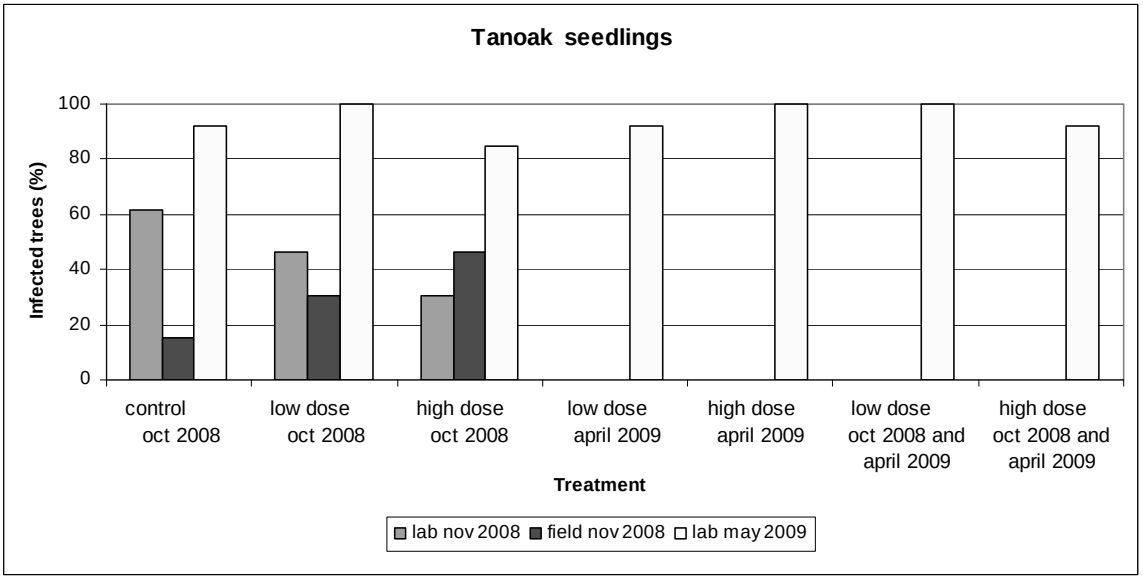

Figure 7—Incidence of infection (percent infected) of tanoak seedlings sprayed with Agri-Fos® at low (0.1 gal/acre) or high (3 gal/acre equivalent) dose, + Pentra-Bark® surfactant , or unsprayed. Seedlings were artificially inoculated with *P. ramorum* by

zoospore spray, or transported to the field for natural infection beneath infected tanoak trees.

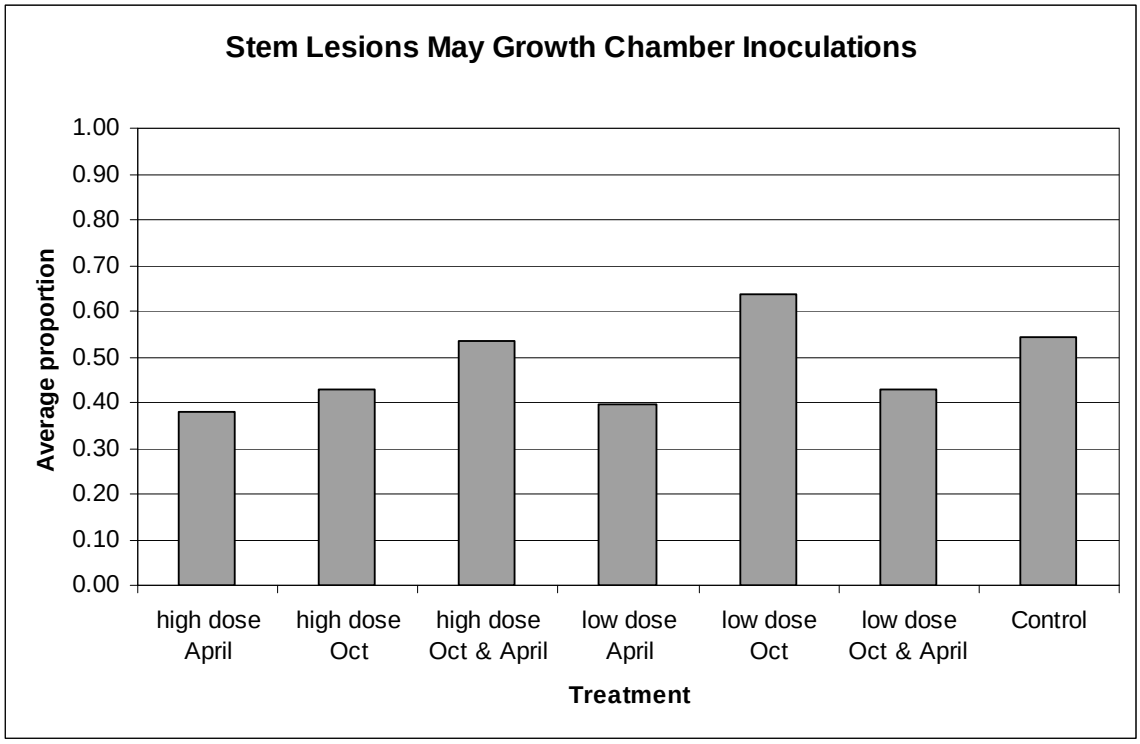

Figure 8—Extent of infection (percent of stem length infected) of tanoak seedlings sprayed with Agri-Fos® at low or high dose, or unsprayed in April 2009. Seedlings were artificially inoculated with *P. ramorum* by zoospore spray or transported to the field for natural infection beneath infected tanoak trees.

Discussion

Interpretation of our Agri-Fos® trials is limited by two factors: lack of a realistic assay for Agri-Fos® uptake of sprayed plants, and inability to test aerial application against natural infection. We are trying to protect tanoak forest trees from initial infection, which occurs through leaves and twigs in the tree crown. Our twig bio-assays of the upper crown, however, show only very small differences in twig lesion lengths between sprayed and unsprayed trees, although our large sample sizes mean that even small differences are often statistically significant. In order to get consistent infection of the twigs, which vary widely in condition, we use a high zoospore concentration (10^4 to 10^5/ml), perhaps overwhelming the Agri-Fos® effect. Wound inoculation of tree boles in the field or in the lab with *P. gonapodyides* or *P. ramorum*, gives larger, more consistently significant treatment differences, demonstrating clearly the uptake of chemical into stems with results comparable to those seen with stem injection. Again, however, Agri-Fos® treatment does not prevent infection entirely, and there has as yet been no demonstration that tree life can be extended appreciably by injection.

Perhaps the small differences in infection we measured by challenge inoculation are sufficient to significantly reduce natural infection of trees in the field. We cannot test aerial application of Agri-Fos® against natural infection of trees, however, because of the ongoing disease control program in Oregon. We cannot leave untreated control plots for comparison with Agri-Fos® treatment. Furthermore, the overall infection rate in the Oregon tanoak forest is very low, necessitating large treatment areas to show differences.

Our aerial application rates are comparable to those used in Western Australia. The bole injection rate, calculated on a per acre basis, is also comparable to our aerial rate (4.15 gal Agri-Fos®/acre). Total helicopter application costs (chemicals, helicopter time, and pilot) in Oregon have been $153/acre for the 3 gal rate and $219/acre for the 6 gal rate.

The seedling results are discouraging. We saw no consistent reduction in infection levels to either artificial inoculation or exposure to natural inoculum, in either trial. The low dose was selected to be equivalent to the standard nursery application rate, and the high seedling dose was the same as the lower of the doses used in the helicopter application. Our assays were not as refined as for the lesion area or twig assays, and we did not have as many replications. Evidently, however, Agri-Fos® does not protect seedlings from *P. ramorum* under these circumstances.

Further complications to the large scale use of Agri-Fos® arise from ownership patterns and land management objectives in southwest Oregon. Industrial landowners have little incentive to protect tanoak that they plan to kill anyway to favor growth of Douglas-fir. Small landowners and rural residential developments do not offer the large treatment areas necessary to make aerial spray applications practical. The conservation management objectives of the federal forest management agencies and large non-profit land conservancies are theoretically more amenable to use of Agri-Fos® to protect stands of tanoak. In these cases, however, cultural and sometimes legal constraints to fungicide use must be overcome.

Eradication Effectiveness Monitoring in Oregon Tanoak Forests[1]

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

Introduction

Phytophthora ramorum was first discovered in Oregon forests in July 2001 where it was killing tanoak (*Lithocarpus densiflorus*) and infecting Pacific rhododendron (*Rhododendron macrophyllum*) and evergreen huckleberry (*Vaccinium ovatum*). At that time, nine infested forest sites were identified ranging in size from 0.2 to 5 hectares, and totaling 16 hectares on non-industrial private forest lands, industrial private forest lands, and federal forest land administered by the Coos Bay District, Bureau of Land Management (BLM). A 23 square kilometer quarantine area was established around the area of infestation. *P. ramorum* has since been the focus of an intense eradication effort on approximately 81 hectares of infested forest land distributed in patches over an area of approximately 155 square kilometers.

Initial eradication treatments involved cutting, piling, and burning infected plants and all nearby potentially infected or exposed host vegetation within a 15 to 30 meter radius buffer zone. Treatment monitoring revealed that the buffer area was inadequate to capture localized spread of the pathogen. In late 2004, buffer zones were increased to a minimum of a 100 meter radius from known infected plants. Monitoring also revealed that newly emerged sprouts from cut infested tanoak stumps were highly susceptible to infection and were maintaining the pathogen on infested sites. A variety of methods to control tanoak sprouting were attempted between 2003 and 2005, including backpack spraying of new sprouts; manually cutting, piling, and burning new sprouts; and stump-top application of herbicides to prevent sprouting. Injecting herbicides (imazapyr or glyphosate) in all tanoak stems 2.5 cm diameter and larger prior to cutting has been used on private, state, and U.S. Department of Agriculture, Forest Service (USDA FS) land since late 2005. On BLM land where herbicide treatments are currently prohibited, sprouts are monitored for disease and are mechanically destroyed if necessary. Upon completion of burning, most sites have been planted with non-host or conifer seedlings.

Monitoring the effectiveness of eradication treatments requires intensive site examinations. The Oregon Department of Agriculture (ODA) has surveyed all treated

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sites for the presence of *P. ramorum* since the initial treatments began in 2001. Some sites have been declared disease-free post-treatment based on inability to recover the pathogen for two consecutive years. However, the large number of treated sites available provides an opportunity to systematically sample most treated sites within a given time frame to:

- describe the overall effect of eradication treatments on the survival and persistence of *P. ramorum* in forest situations (in soil, vegetation, and streams);
- describe the amount and species of vegetation that either survived the treatments or colonized the treated sites following treatment; and
- compare the effects of herbicide (non-federal, state parks, and USDA FS lands) and non-herbicide (BLM) treatments on vegetation and pathogen survival.

In 2008 we began the first of a two year project to revisit most treated sites to sample soil and vegetation in plots centered on stumps of known infected trees.

Methods

Plots were established around stumps of known infected trees. At each plot a 1 liter composite soil sample was collected from within 2 meters of the known infested stump. An additional nineteen 1 liter soil samples were collected across a 0.02 hectare circular plot. Samples were split; a 0.5 liter portion of each sample was assayed for *P. ramorum* as quickly as possible by the Oregon State University (OSU) lab and the remaining 0.5 liter soil sample was placed in cold storage and sampled approximately one month after collection by the ODA lab. Soil samples were wetted and baited using either rhododendron or viburnum leaf pieces. *P. ramorum* was confirmed by PCR and culturing of baits.

On the same 0.02 hectare plot, tanoak stumps were tallied by condition (live with sprouts or dead), and diameter and standing trees of all species greater than 12 cm diameter were also tallied by species and diameter. Host vegetation was examined for symptoms of *P. ramorum*. A minimum of five vegetation samples were collected from each plot and sent to the labs for confirmation of *P. ramorum* infection via ELISA, PCR, and culture.

A 0.008 hectare circular plot centered on the infested stump was used to describe percent cover of shrubs and forbs. Tree seedlings and saplings were also tallied on this smaller plot.

Preliminary Results

A total of 119 plots that had received eradication treatments between 2001 and 2007 were sampled in late 2008 to spring 2009. Based on one round of soil baiting only (OSU/immediate baiting) we did not recover *P. ramorum* in soil or vegetation from 70 (59 percent) of the 119 plots sampled. Thirty-seven (31 percent) of the plots yielded cultures of *P. ramorum* from soils only, eight plots (7 percent) yielded *P. ramorum* from soils and vegetation, and on four (3 percent) plots, *P. ramorum* was recovered only from vegetation. On those plots where *P. ramorum* was baited from soil, recovery was generally low; on 22 of 45 plots, only one of 20 soil samples

yielded *P. ramorum*. When all soil samples were combined, 96 percent of 2380 soil samples collected were negative for *P. ramorum* based on the first round of baiting. Fifteen of the 88 (17 percent) *P. ramorum*-positive soil samples were collected adjacent to the identified infested stump.

Twelve (10 percent) of the plots had vegetation samples that were positive for *P. ramorum.* Eight of these plots (75 percent) also had soil that yielded *P. ramorum.* A total of 19 vegetation samples out of 595 (3 percent) collected were positive for *P. ramorum*. All positive samples were tanoak; most of the diseased material was collected from tanoak basal sprouts.

No *P. ramorum* was recovered from plots on sites treated in 2002; all other treatment years yielded some *P. ramorum.* Most of the *P. ramorum*-positive plots were associated with more recent treatment years (2006 and 2007).

Some herbicide use was associated with 106 of the plots sampled; 13 plots had no herbicide treatment history. Sample sizes are decidedly unequal, nonetheless, where herbicide was used, 62 percent of the 106 plots yielded no *P. ramorum*, in 31 percent of plots *P. ramorum* was recovered in soil only, 5 percent of plots yielded *P. ramorum* in soil and vegetation, and in 2 percent of the plots *P. ramorum* was recovered from vegetation only. Where herbicide was not used, 31 percent of the 13 plots yielded no *P. ramorum,* in 31 percent of plots *P. ramorum* was recovered in soil only, 23 percent of plots yielded *P. ramorum* in soil and vegetation, and in 15 percent of the plots *P. ramorum* was recovered from vegetation only.

The next steps in this continuing study will be to evaluate the results of soil baiting done after cold storage and to analyze the vegetation data. We plan to establish additional plots in 2009 and 2010 as well as revisit a subset of plots visited in 2008 and 2009.

Assessing Methods to Protect Susceptible Oak and Tanoak Stands from Sudden Oak Death[1]

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

Abstract

Landowners and managers have been seeking ways to protect susceptible oak (*Quercus*) species and tanoak (*Lithocarpus densiflorus*) from sudden oak death (SOD) caused by *Phytophthora ramorum.* Because disease epidemiology differs between tanoaks and susceptible oaks, we are testing different control strategies appropriate for different forest types. The disease management studies described here test whether various management techniques will be effective when applied in the field at the stand scale.

In tanoak stands, we are testing applications of potassium phosphite (Agri-Fos®) in contiguous blocks of trees ranging from about 250 m^2 to 1.3 ha. Our initial study plots were established in December 2005 in Sonoma County; the most recent and largest plots were established in San Mateo County in 2007 and 2008. In all plots, we have used bark spray applications (Agri-Fos® diluted 1:1 with water + 2.5 percent Pentra-Bark® surfactant by volume) with rates scaled to stem cross-sectional area (fig. 1). The curve is linear for stems up to 30.5 cm diameter at breast height (DBH). For larger stems, the curve uses a quadratic formula to deliver a more constant dose of phosphite per unit volume of sapwood (Swiecki and Bernhardt 2007). We also shifted the application zone higher on the stem (up to 6 m height) to enhance uptake. Agri-Fos® was applied to trees in treated plots twice in the first year with a 6 month retreatment interval and with a 12 month retreatment interval thereafter. At one location, stem injection applications using two different injection systems were added for comparison purposes in 2008.

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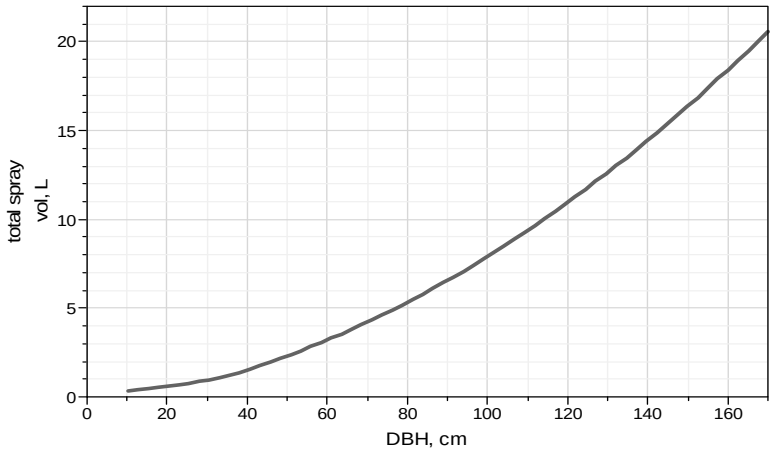
Figure 1—Curve showing volumes of diluted Agri-Fos® spray solution used for stems of varying diameters.



Only a few plots contained small trees or saplings of California bay laurel (*Umbellularia californica*), which were removed at the time that plots were established. In all sprayed plots and matched control plots (thinned controls), small diameter tanoak stems (generally <5 cm DBH) were also removed prior to treatment. Small stems are difficult to treat and evaluate for disease due to foliar phytotoxicity from the phosphite/surfactant spray solution. In two locations where we removed a significant amount of understory tanoak, we set up a second control plot (non-thinned control) with no understory tanoak removal. The plots without understory tanoak removal are included as a check to determine whether removal of understory tanoaks alone can affect disease development within the plot.

To date, SOD-affected trees are lacking or occur at very low percentages in all but the oldest plots, where no significant differences due to treatment have yet been detected. Incidence of SOD ranges from 2 to 27 percent in these plots (table 1). Most of these infections were likely initiated in the last two wet spring seasons, 2005 (prior to treatment) and 2006 (after the initial application). Monitoring conducted in plots in spring 2007, 2008, and 2009 failed to detect *P. ramorum* inoculum, indicating that disease pressure has been very low for the past 3 years.

Table 1—SOD incidence and mortality of tanoak stems attributed to *P. ramorum* and other factors observed 42 months after initial treatment in December 2005; plots are located in northwest Sonoma County; thinning refers to removal of small understory tanoak

Location	Plot	Treatment	Live stems at start of study	Percent of stems with likely *P. ramorum* canker	Percent overall mortality	Percent mortality attributed to *P. ramorum*
BL	BL3	Agri-Fos+thin	57	1.8	1.8	0
	BL4	thinned control	57	1.8	7.0	1.8
	BL5	non-thinned control	56	7.1	5.4	5.4
SF	SF1	Agri-Fos+thin	63	27	14.3	12.7
	SF2	thinned control	61	13.1	1.6	1.6
	SF6	non-thinned control	72	16.7*	8.3	8.3

*Three percent of stems in this plot were symptomatic at the start of the study.

In oak stands, where California bay laurel is the primary source of *P. ramorum* inoculum, we are testing local and area-wide removal of bay laurel near susceptible coast live oak (*Quercus agrifolia*), California black (*Q. kelloggii*), and Shreve oak (*Q. parvula* var. *shrevii*) as a disease management technique. At one location with high-value coast live oaks along a seasonal creek, bay laurel removal is being combined with phosphite application (by spray or injection) because the extent of bay laurel removal was limited due to other management considerations.

We established plots to study bay laurel removal in localized zones around individual oaks (minimum bay laurel foliage to oak trunk clearance = 2.5 m) at five locations in Sonoma, Napa, and Solano Counties in 2007. At each site, bay laurel neighborhoods around oaks and oak disease status were assessed prior to treatment. We identified 49 matched pairs of trees with similar bay laurel neighborhoods and then removed bay laurel around one member of each pair, leaving the untreated member as a control. Bay laurel neighborhoods were reassessed immediately after treatment and annually thereafter. To date, due to low inoculum pressure associated with drought conditions, no differences have developed between treated and untreated plots.

In two locations in San Mateo County, we initiated studies of area-wide removal of all bay laurel within patches as large as 2.75 ha in 2009. Control plots without bay laurel removal were established adjacent to the treated plots. Bay laurel neighborhoods and disease status for a sample of the oaks in each plot were assessed prior to treatment. Bay laurel neighborhoods in the treated plot were reassessed shortly after bay laurel removal was completed.

Sprout regrowth from cut bay laurel stumps has not been very vigorous in the first year after cutting, especially in relatively dry sites. Evaluations in June 2008 showed that browsing by deer and other animals was highly effective at reducing bay laurel sprout growth. At four of five locations (Sonoma, Solano, Napa Counties), average maximum bay laurel sprout height was 37 cm or less one year after cutting. Only minimal browsing occurred at the fifth location. At this location, average bay laurel sprout height was 80 cm and the tallest bay laurel sprout was 1.3 m after one year. Basal diameters of sprouts averaged about 0.78 cm and were easily removed using loppers or an axe. Very few of the pruning cuts made to remove bay laurel branches from larger stems gave rise to epicormic sprouts. At the two San Mateo locations, stumps were sprayed with glyphosate (20.5 percent) immediately after cutting to suppress sprout growth. By 6 months after treatment, sprouts were lacking on almost all treated stumps.

Continued monitoring is needed to determine the efficacy of both phosphite and bay laurel removal treatments. However, these studies show that it is feasible to implement these disease management methods at various spatial scales to protect tanoaks and oaks from SOD.

Acknowledgments

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Forest Treatment Strategies for *Phytophthora ramorum*[1]

Yana Valachovic,[2] Chris Lee,[2] Jack Marshall,[3] and Hugh Scanlon[4]

Abstract

Although there is no known cure or preventative on a landscape scale for sudden oak death (SOD), the plant disease caused by *Phytophthora ramorum*, a variety of management options has been tested with the goal of developing an integrated program of treatment for the pathogen. This paper presents a first attempt to gather together individual management trials into an overall decision-making tool for landowners contemplating treatments for the disease. It conceptualizes these treatments as a matrix that matches available strategies—some of which are still substantially untested—to management goals for properties or landscapes of varying sizes. The major goals we envision for landowners who are making decisions about *P. ramorum* treatments include 1) minimizing property impacts from the pathogen when it is already established on a property; 2) strategically protecting particular geographic locations, areas of high-quality oak and tanoak resources, or "islands" of old-growth oak and tanoak; and 3) suppressing *P. ramorum* inoculum and limiting its spread on a landscape (or larger) level. For each goal, we consider a number of possible treatment approaches. A key principle for landowners to keep in mind when considering strategies for managing *P. ramorum* is that all treatments should complement long-term goals for the property. In general, the action that should be taken in an area should be appropriate to the size of an epidemic; the most effective treatment programs involve early intervention.

Introduction

There is no known cure or preventative on a landscape scale for sudden oak death (SOD), the plant disease caused by *Phytophthora ramorum*, an invasive introduced pathogen that has been killing trees in California and Oregon since the mid-1990s. However, a range of management options exists for fulfilling a number of *P. ramorum* management goals, from alleviating the impacts of tree mortality on a given property to protecting particular stands from infestation. This paper presents a range of those options in the form of a matrix (table 1) that matches them to typical land management goals related to *P. ramorum* in the hope that land owners and land managers might find it useful as a decision-making tool.

This systematic categorization of treatments is not exhaustive and can be added to or adapted to individual land managers' needs. Systematically organizing and presenting them in this manner may help counter a persistent perception that in areas where *P. ramorum* is established, or in areas along the California coast in general, there is "nothing that can be done." The discussion here centers around and proceeds from the understanding that effective treatment depends not only on the motivation to manage, but also on a clear articulation of management goals.

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Setting Goals and Objectives

Effective management of any element of forest structure or function—and this includes disturbance agents like tree pathogens—starts within an understanding of the larger picture in two respects: (1) What are my goals for the forest and/or individual trees on my property? and (2) How does that fit into the context of the ecology of the forest in question? How does typical forest stand development happen over time here, and what are the individual components of that development?

Determining management goals for the landscape can be a daunting task for the landowner or land manager (henceforth "manager"), but it is an essential first step for planning. The condition of any given property in California is most likely the result of past management actions, and managers have many factors to consider, including erosion and water quality issues, sources of fuel, wildlife, aesthetics, and other environmental values (Giusti and Harris 2007). Managers may want to manage their forested properties for occasional or sustained financial return from timber or grazing; maintenance of wildlife populations or habitat for sensitive species; recreation such as horse or ATV riding or camping; scenery; home sites; aesthetics; demonstration of landscape management techniques; preservation of cultural resources or historical sites; or a variety of other purposes.

Understanding the ecology of local forests provides essential knowledge that narrows the range of management actions available to the manager. Managers should understand how the physical characteristics of the landscape affect plant growth and succession; how vegetation structure influences fire risk and erosion hazards; which wildlife species depend on which other species (plant, animal, and fungal); which species are native and which are introduced; and so on. Conducting an inventory and assessment of the property and its variety of habitat conditions, while time-consuming, is important to establish informed criteria for making management decisions. Managers can consult trained professionals, such consulting foresters or rangeland managers, University of California (UC) Extension advisors, and California Department of Forestry and Fire Protection (CAL FIRE) service foresters for help in understanding the range of options.

Putting *Phytophthora ramorum* in Context

Returning to the problem of managing *P. ramorum*, then, becomes clearer once the manager has articulated general management goals and limited the management options available on the property. However, it also becomes more complex as the manager realizes that he or she must fit *P. ramorum* into a context of other, simultaneous management goals. Fortunately, many of these management goals are complementary to the options available for *P. ramorum* management. Thinning susceptible host trees such as tanoaks (*Lithocarpus densiflorus*) to discourage pathogen persistence, for example, may hasten the development of historically Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) dominated forest stands into a late-seral condition characterized by a stratum of very large, tall Douglas-firs over a secondary canopy of a few large hardwoods—desirable for many publicly managed forests. Improving roads to control erosion often has the side benefit of eliminating muddy areas, lessening the chance that *P. ramorum* inoculum will be moved off-site on vehicle tires. Constructing shaded fuelbreaks to provide fire control points and reduce fuel ladders along strategic roads and ridgetops can open up the stand, reducing both humidity (which *P. ramorum* needs to survive and reproduce) and absolute numbers of *P. ramorum* hosts.

Phytophthora ramorum management must also be seen within the context of larger regional vegetation ecology. The palette of management actions available in Humboldt County, where tanoak is the primary host to sustain lethal infections, differs from the actions available in coast live oak (*Quercus agrifolia*) dominated areas of Monterey County. Options in both of these example areas will differ from those available in southwestern Oregon, where the presence of California bay laurel (*Umbellularia californica*) does not drive disease spread in the same way that it does in California.

Testing Management Options

A number of researchers have been testing a variety of management options for *P. ramorum* (Garbelotto and Schmidt 2009, Garbelotto and others 2007, Goheen and others 2002, 2004, Kanaskie and others 2006, Swiecki and Bernhardt 2007, Valachovic and others 2008). Taken together, and keeping in mind the regional differences mentioned above, they form the beginnings of an integrated, adaptive program to learn how best to treat forest stands infested by *P. ramorum*. These treatments are in a variety of stages of testing, and even ones that have been shown to be effective continue to undergo revision as our knowledge of *P. ramorum* biology advances.

Table 1 summarizes some representative available treatments (including treatments both tested and untested) according to the primary *P. ramorum* management goal on that property. For simplicity, we have defined three common management goal scenarios: (1) minimizing property impacts from SOD; (2) strategic protection of tanoak islands, old-growth tanoak, or particular geographic areas; and (3) suppression of *P. ramorum* and limitation of spread. The first scenario presupposes active disease on the property, so that managers want to limit secondary problems accruing to tree mortality, such as safety and fire risks. The third presupposes concern for an area landscape larger than most individual properties. The Oregon eradication attempts belong to this category.

The manager should keep in mind at least two related points when reviewing the list presented here. First, the options presented here do not exhaust the range of treatment options for *P. ramorum* that have been proposed, are being tested, or have been shown to be effective in certain situations. Some treatments that are unique to a limited set of site variables (such as implementing host-free barrier zones) have not been presented here. Second, the landscape where many of these treatments have been tested is the north coast of California, where tanoak is the primary species to sustain lethal infections and large tracts of uninfested property exist that both represent ideal habitat for *P. ramorum* infestation and contain high-quality resources that warrant large-scale protection efforts. Because of this, our discussion limits itself to the range of site conditions present on the north coast. However, most of these treatments have analogues in the other regions of California and Oregon where *P. ramorum* is present or can be adapted to fit those regions.

Table 1 Matrix of *P. ramorum* management goals and possible treatments, with lead researchers for treatments

Goal	Minimizing Property Impacts from Sudden Oak Death	Strategic Protection of Oak and Tanoak Islands, Old-Growth trees, or Particular Geographic Areas	Suppression of *Phytophthora ramorum* and Limitation of Spread
Treatment	Dead tree removal[¶]	Manual removal of California bay laurel only[*,†]	Manual removal of bay laurel and tanoak (+/- prescribed underburning)[*]
	Reforestation	Agri-Fos® application[*,†,‡]	Modified fuel hazard reduction removal (+/- California bay girdling)[*]
	Maintain some tanoak with thinning (manual or by Agri-Fos®)[¶]	Combination of manual removal of bay laurel and Agri-Fos® application[¶]	Herbicide host removal (California bay laurel and tanoak)[*]
	Combination treatments to address site specific goals		

*Tested by Y. Valachovic and others in Del Norte, Humboldt, and/or Mendocino Counties
†Tested by T. Swiecki and others in Bay Area and surrounding coastal California area
‡Tested by M. Garbelotto, D.J. Schmidt, and others in Bay Area and surrounding coastal California area
§Tested by numerous researchers
¶Still untested

Which Species to Treat?

Most treatments target California bay laurel and tanoak because these are the two host species known to most readily support *P. ramorum* sporulation in California wildlands (Davidson and others 2001, Davidson and Shaw 2003, Maloney and others 2005), allowing for subsequent spread to other trees . Little is known about the ecology of these two species or their appropriate management. We do know that both have increased in abundance over the past century because of fire suppression and the tanbark industry (Meentemeyer and others 2008, Stuart and Stephens 2006, Tappeiner and others 1990). Each species has an important ecological role; an integrated approach to disease management will not advocate for widespread removal of either species.

Testing Treatment Effectiveness

Many treatment studies are still in progress, with results that are more suggestive than conclusive. A number of methods exist to assess treatment effectiveness, most of them based on monitoring treated stands for pathogen presence at various times after treatment. These methods include (1) periodic, usually annual, return visits to established monitoring plots to survey for the beginnings of new disease symptoms; (2) spore traps consisting of water-filled buckets with floating rhododendron or California bay laurel leaves to bait any pathogen spores that move through the air and fall into the buckets in wet winter and spring weather; and (3) baiting *P. ramorum*

spores from soil collected at the treatment site. However, managers should not forget the qualitative aspects of evaluating treatments, including the satisfaction of the other, complementary management goals mentioned earlier in this paper. For example, at a ranch in Mendocino County where dead and infected trees are removed yearly to improve aesthetics and reduce fire hazard, the owners assume that *P. ramorum* will persist on-site, even if at low levels, so the look of the forest, the wildlife it attracts, and the alleviation of safety hazards serve as their metrics for evaluating the treatments implemented by the property foresters.

Treatments

Goal: Minimizing Property Impacts from Sudden Oak Death

These strategies seek to cope with the continued presence of *P. ramorum* by addressing its impacts in such a way that fire hazard is lessened, the forest is revegetated appropriately, and aesthetic values are maintained.

Dead Tree Removal–

Various studies designed to answer questions about the contribution of SOD to hazardous fuel amounts and configurations are underway (Lee and others, these proceedings). These answers could help set guidelines for how long the fuel risk will be of concern and how aggressively landowners should manage dead tree removal on their properties. Treatments that remove dead trees also help to alleviate safety (among other) concerns.

Reforestation–

Landowners throughout California have numerous questions about how and what to replant to replace tanoaks and true oaks killed by *P. ramorum*, but little research has been done to answer them. In each situation, it is also important to know what regenerates naturally after *P. ramorum* kills on-site oaks and tanoaks. Appropriate replanting will vary depending on the property manager's future desired condition for the landscape and whether new seedlings will survive or be killed by new waves of *P. ramorum* without removal of nearby infectious California bay laurel trees.

Tanoak Component Retention–

Managers might want to maintain some specific numbers of tanoak trees, or particular individual trees, on site for several reasons, including maintaining complexity of canopy layers, preserving old-growth tanoaks, or supporting wildlife. This sort of treatment may be more warranted in tanoak stands than in true oak stands because *P. ramorum* is more likely to kill a greater proportion of the stand in the case of tanoak (Garbelotto and Schmidt 2009) and because tanoak forests are generally much more dense than true oak forests or woodlands. Focusing on key specimen trees, thinning small neighbors of large trees that the manager desires to retain, or treating specimen trees with the systemic fungicide Agri-Fos® (see Agri-Fos® Application in Forests, below) long in advance of pathogen arrival at the site are all options to achieve this goal. Trials now being designed in the north coast will be conducted at varying levels of tanoak retention to test how much tanoak can be left on site while still providing protection from *P. ramorum.*

Goal: Strategic Protection of Tanoak Islands, Old-Growth Tanoak, or Particular Geographic Areas

If property managers wish to protect certain areas or groves of tanoak or true oak trees, they can introduce specific chemical barriers (see Agri-Fos® Application in Forests, below) for tanoaks or true oaks (the main lethal hosts of the pathogen) or possibly remove bay laurel (the main reproductive platform for the host). In California, bay laurel consistently becomes infected in a given area months to years before tanoaks begin to die. While widespread removal of California bay laurel is not advocated, it may be possible to protect areas by some strategic removal, especially where California bay laurel density is low and/or where the trees are just becoming established.

Manual Removal of California Bay Laurel Only–

Since California bay laurel is the main transmitting host, it may be possible to slow the spread of the disease by removing California bay laurel only. This is similar to the approach utilizing removal of both California bay laurel and tanoak, except that because smaller numbers of trees are felled, labor and costs are much less. It is sometimes possible to leave entire trees to decay on the ground unprocessed, which can reduce costs even more, although lopping and scattering the fine branches is recommended to speed decomposition and alleviate fire risk. Again, California bay laurel removed in this manner will sprout. As mentioned above, it is not usually necessary to remove all California bay laurel trees on a given property; rather, *strategic* removal is advised. California bay laurel removal projects should take advantage of the distribution and sizes of California bay laurel trees across the landscape (and in relation to the locations of oaks and tanoaks). The manager may find that it is too expensive or environmentally costly to remove large, old California bay laurels.

Agri-Fos® Application in Forests–

The systemic fungicide Agri-Fos® has been shown in the laboratory to be effective in preventing infection in uninfected tanoak and oak trees to which the fungicide is applied (Garbelotto and others 2007, Garbelotto and Schmidt 2009). Agri-Fos® is the trade name for phosphonate, a neutralized form of phosphorous acid (H_3PO_3). Although it was initially investigated as a potential fertilizer, phosphonate soon became recognized for its systemic fungicidal qualities (Bender 2005). Systemic fungicides work by traveling through the tree's transport system to all parts of the tree; phosphonate fungicide stimulates the production of defensive chemical compounds and thus the tree's resistance to pathogen invasion and pathogen growth. Agri-Fos® has mostly been used until now to protect individual landscape trees of concern. Current studies are transferring the existing laboratory- and orchard-based trials outdoors to test the efficacy of the fungicide on larger groups and landscapes of tanoak and oak trees. The efficacy of Agri-Fos® against *P. ramorum* lasts for about 2 years (Garbelotto and Schmidt 2009), necessitating repeated booster treatments indefinitely.

Combination of Manual Removal of California Bay Laurel and Agri-Fos® Application–

While not field tested, this combination could potentially suppress *P. ramorum* sporulation more than either technique alone. This may be an effective technique for application to stands of trees in areas where a strategic barrier is desired, such as the outer edges of the stands or on ridgetops.

Goal: Suppression of *P. ramorum* and Limitation of Spread

In wildlands where *P. ramorum* presence is very limited and the pathogen infests small, isolated areas, a set of tools exists to attempt to suppress spore production, modify the environment to discourage pathogen persistence, and make long-range pathogen "jumps" to other, uninfested areas more unlikely.

Manual Removal of Tanoak and California Bay Laurel--

This treatment, as it has been tested, focuses on the removal of both major hosts that support *P. ramorum* sporulation in north coast forests. It involves cutting all tanoak and California bay laurel trees and piling and burning the foliage and small branches. Large branches and trunk wood can be left on site or removed for use as firewood (if done with caution and under guidance so that potentially infected materials do not leave the generally infested area). This is one of the most costly ($1500 to $3000/acre) (Valachovic and others 2008), labor-intensive, and time-consuming silvicultural approaches to controlling SOD. It is also the most thorough, especially when combined with prescribed underburning to remove seedlings and infected foliage. Without further treatment, tanoak and California bay laurel stumps will re-sprout and grow vigorously.

Modified "Fuel Hazard Reduction" Removal–

This approach mimics the shaded fuelbreaks created by fuel hazard reduction projects in strategic locations around the American West. A large proportion of small trees and underbrush are removed to increase spacing between trees, clear out the understory, and reduce fuels that could move fire vertically into tree canopies. It is hoped that this treatment might reduce humidity in the forest understory and so make it more difficult for the spores of the SOD-causing pathogen to persist. Along with some unspecified proportion of tanoak trees, this treatment should attempt to remove all California bay laurel trees within the treatment area. Costs vary by stand condition, but should generally be less than the removal of all tanoak and California bay laurel trees as described in the prior prescription. One difficulty is that large California bay laurel trees, which are not normally removed in a typical shaded fuelbreak situation, can be costly and time-consuming to cut down and process; killing the tree in place by girdling has been tried, but without success. The public is already generally accustomed to this mode of forest management, which could render it more acceptable to use than some other techniques.

Herbicide Host Removal–

Killing tanoak and California bay laurel with herbicides is much cheaper ($200 to $250/acre) and less time-consuming than manual removal. It also carries the advantage of keeping the stumps of this host from re-sprouting, thus removing the possibility that those sprouts will be reinfected (Kanaskie and others 2006). However, it will increase fuel hazard as standing trees die, retain their foliage for a short while, and dry out before falling and decomposing. To alleviate this concern, it is also possible to manually cut and process trees and then treat the stumps with herbicide to prevent resprouting. Herbicide use is not acceptable to all landowners. Additionally, the standard herbicide treatment used in forestry applications (imazapyr) has proven insufficient to rapidly defoliate California bay laurel; follow-up herbicide efficiency trials are underway in Humboldt County.

Summary

Other treatments or treatment combinations may become possible as new ideas present themselves. All treatments should complement the long-term goals of the landowner for the property and can include other silvicultural elements such as replanting or pruning. The treatments presented here are unlikely to eradicate the SOD pathogen; such an eradication program requires sustained effort and repeated treatment entries into forest stands over many years. In general, the action that should be taken in an area should be appropriate to the size of the epidemic; the most effective treatment programs involve early intervention (Gilligan 2007). All actions designed for SOD management should be undertaken after gathering appropriate information, seeking technical assistance and advice, thinking carefully about the likelihood of success, and weighing the possible benefits to resources against the possible costs.

Acknowledgments

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Fire and Social Impacts

Effects of Sudden Oak Death on the Crown Fire Ignition Potential of Tanoak (*Lithocarpus densiflorus*)[1]

Howard Kuljian[2] and J. Morgan Varner[2]

Abstract

In the face of the sudden oak death (SOD) epidemic, decreasing foliar moisture content (FMC) of tanoak (*Lithocarpus densiflorus*) has land managers, fire managers, and property owners concerned with the increased possibility of crown fire in affected areas. A need exists to link local SOD-affected foliar moisture content (FMC) values and current FMC data to decision support tools, allowing managers to better predict crown fire in areas where SOD-affected tanoaks are prevalent. We tracked FMC of live (uninfected) tanoaks, *Phytophthora ramorum*-infected tanoaks, dead tanoaks, and surface litter for 12 months. We found that FMC values differed significantly among the three categories of uninfected, *P. ramorum*-infected and dead leaves. FMC of live tanoaks averaged 82.3 percent for the year whereas FMC of infected tanoaks had a lower average of 77.8 percent (ANOVA, $P = 0.04$). Dead trees had a significantly lower FMC, averaging 12.3 percent (ANOVA, $P < 0.01$) for the year. During fire season (June to September), dead tanoak FMC reached a low of 5.8 percent, with no significant difference between dead canopy fuels and surface litter (ANOVA, $P = 0.44$). Application of low FMC values to a crown ignition model results in extremely high crown base height (CBH) values to escape crown ignition. Remote estimation of dead leaf moisture using 10-hour fuel moisture shows promise. Preliminary results indicate a strong relationship between remote automated weather station (RAWS) 10-hour fuel moisture data and the FMC of dead leaves ($R^2 = 0.78$, $P < 0.01$).

Results from this on-going study will aid the decision support process for fire managers in SOD-affected areas and may also be applicable to conditions in other ecosystems where diseases and insect epidemics have altered forest canopy fuels.

Introduction

As sudden oak death (SOD), caused by *Phytophthora ramorum*, continues its spread throughout coastal California, changes in foliar moisture content of tanoaks (*Lithocarpus densiflorus* [Hook. and Arn.] Rehd.) has land managers, fire managers, and property owners concerned with the possibility of crown fire in forests that have been largely crownfire resistant. At this point, no research has been completed to determine if there is an increase in crown fire potential resulting from *P. ramorum*-infected and/or killed tanoaks.

Crown fire potential can be predicted using fire modeling software such as FVS Fire and Fuels Extension (Reinhardt and Cookston 2003), Crown Mass (Carlton 2005), BehavePlus (Andrews and others 2008), FARSITE (Finney 2004), and NEXUS

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(Scott 1999, Scott and Reinhardt 2001). All of these models use Van Wagner's (1977) crown fire initiation and spread model. Aside from weather and topography, this model is dependent on three principle factors: surface fireline intensity (related to flame length); crown base height (CBH); and foliar moisture content (FMC) (Van Wagner 1977).

Relative to flame length and CBH, the effect of FMC has been considered minor, hence the majority of fire modeling programs recommend using a default foliar moisture value = 100 percent (Scott and Reinhardt 2001, Cruz and others 2006). Given that infected tanoaks die and retain their leaves for months to years post-death (D. Rizzo, professor of plant pathology, University of California at Davis, personal communication 2007), FMC values fall far below the normal range of expected FMCs, reaching values as low as 5 percent (Kuljian and others, in press). What is needed is a means to link localized SOD affected FMC values and currently available FMC data to decision support tools so that managers can better predict crown fire in areas where SOD-affected tanoaks are a considerable forest component.

The purpose of this study was: (1) to measure and track the FMC of healthy tanoaks, tanoaks infected with *P. ramorum*, and dead tanoaks with leaves attached for 1 year; (2) to evaluate the effects of low FMC to crown fire potential of using Van Wagner's (1977) crown fire model to predict crown fire ignition with these values; and (3) to investigate the potential of using 10 hour fuel moisture data obtained from remote automated weather station (RAWS) to predict the FMC of dead tanoak leaves.

Methods

Foliar Moisture

In March 2008, we began tracking FMC for live tanoak leaves (uninfected), live *P. ramorum*-infected tanoak leaves, dead tanoak leaves, and surface leaf litter. Individual tanoak trees were selected in an area of known *P. ramorum* infestation on Cal Fire Eel River Camp and adjacent properties near Redway, California (40° 08' 29.12" N, 123° 49' 29.80" W) where SOD has been known to exist since 2004 (Y. Valachovic, Humboldt County Cooperative Extension Agent, University of California, personal communication, 2007). A total of 25 tanoak trees (eight live uninfected tanoaks, 10 live *P. ramorum* infected tanoaks, and seven standing dead tanoaks with leaves on) were selected for monthly sampling. Each sample tree was sampled each month for 12 months (March 2008 to February 2009). All foliar samples were removed from the lower 1/3 of the canopy on the south aspect of each tree (Agee and others 2002) between 1300 and 1600 hours to minimize possible diurnal variation (Philpot 1965). Collected foliage was sealed in polyethylene bags, weighed wet, then oven-dried at 70 °C until no further weight loss was evident (typically 48 hours).

Data were analyzed across three categories of infection (non-infected, infected, and dead) using repeated measures ANOVA. Additionally, surface litter moisture was compared to dead leaf moisture for each month. For each collection date, the moisture content of each category was compared using Tukey-Kramer post-hoc means separation. All statistical tests held the α level of 0.05.

Crown Ignition

Critical crown base height (CBHt) for tanoak was calculated using a reformulation of Van Wagner's canopy ignition equation:

$CBHt = [FLI(1/1.5)] / [(0.010)(460+26*FMC)]$
where: CBHt is the crown base height (in meters) below which ignition is probable given a predicted flame length. Values of FMC were derived from the sample data collected. We used flame lengths of 0.5, 1, and 2 m based on fuel loading and resultant fire behavior typical of these forests.

RAWS Data

The relationship between data from local RAWS 10-hour fuel moisture and dead leaf moisture (FMC_{dead}) was examined using data from the RAWS unit at the Cal Fire Eel River Camp, located less than 1 km from all dead tanoak sample trees. RAWS 10-hour fuel moistures were averaged for four different time periods starting at 1200 hours. Linear regression analysis was used to verify the most significant window of time during the day as a predictor of FMC_{dead}. The response variable (FMC_{dead}) was transformed using natural log to normalize the residuals.

Results

FMC Across Tanoak Infection Status

The FMC values differed significantly among the three categories of uninfected, infected, and dead trees ($P < 0.01$). The 1-year mean FMC for uninfected tanoak leaves was 82.3 percent (SE = 1.5 percent) with a range from 79.5 percent in May to 86.7 percent in December. The FMC of the *P. ramorum*-infected tanoaks maintained a lower average of 77.8 percent (SE = 1.3 percent; $P = 0.04$) across the sampling period with a range from 72.7 percent in September to 83.0 percent in December. The FMC of dead leaves was significantly lower than both uninfected and infected leaves for all months with a mean of 12.6 percent across the 12-month sampling period (SE = 1.4 percent, $P < 0.01$; fig. 1).

Surface Litter

Fuel moisture of surface leaf litter varied significantly among months with a high of 40.3 percent in December to a low of 7.1 percent in July ($P < 0.01$). Across the year, leaf litter moisture was significantly different than dead leaf FMC; however, in fire season months (June to September), there was no significant difference between dead leaf FMC and surface litter ($P = 0.44$; fig. 2).

CBH_t

These low values of FMC in *P. ramorum*-killed tanoaks resulted in dramatic changes in CBH_t (fig. 3). Critical crown base height rises radically below an FMC of 70 percent. For example, given a 1 m flame length, CBH_t for an FMC of 80 percent is 1.6 m. As FMC drops to 5 percent, the CBH_t increases to 6.9 m. Note that for values below 70 percent FMC, a transition zone is included showing an assumed error of ±5 percent sequentially for every 10 percent decrease in FMC.

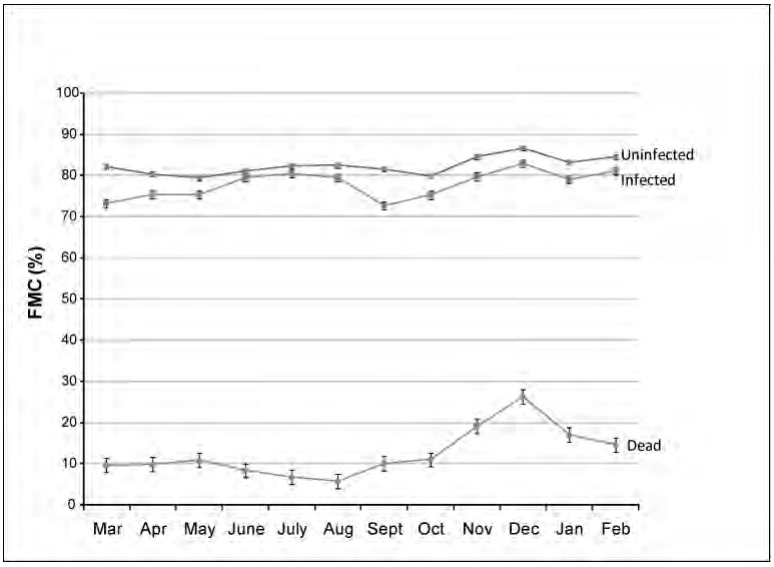

Figure 1—Monthly leaf moisture of Eel River Camp tanoaks across three categories of infection status: Uninfected, *P. ramorum*-infected, and Dead. Mean FMC of *P. ramorum*-infected and non-infected tanoaks did not differ more that 10 percent for the 12-month sampling period. Mean FMC of dead tanoaks was significantly lower than infected and non-infected groups (SE = 1.4 percent, P < 0.01).

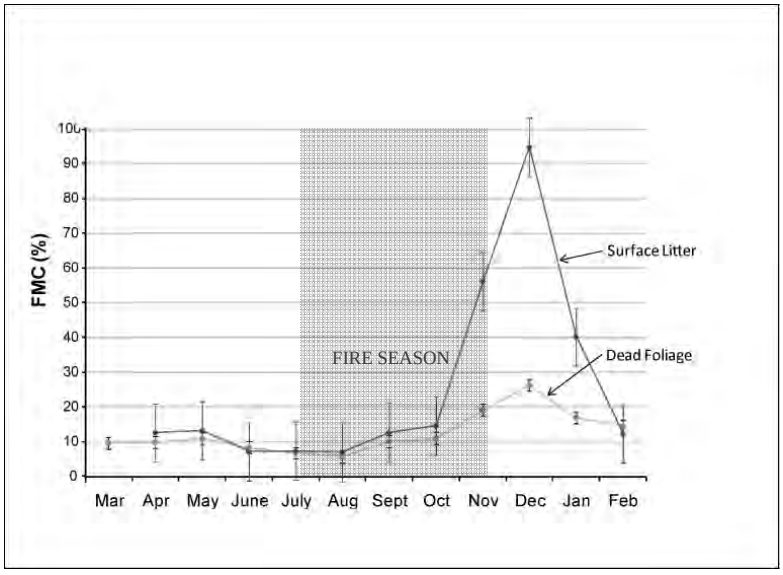

Figure 2—Monthly dead leaf moisture of tanoaks and surface litter moisture of Eel River Camp tanoaks. Months June through September show no significant difference in moisture content (SE = 0.6; P = 0.44).

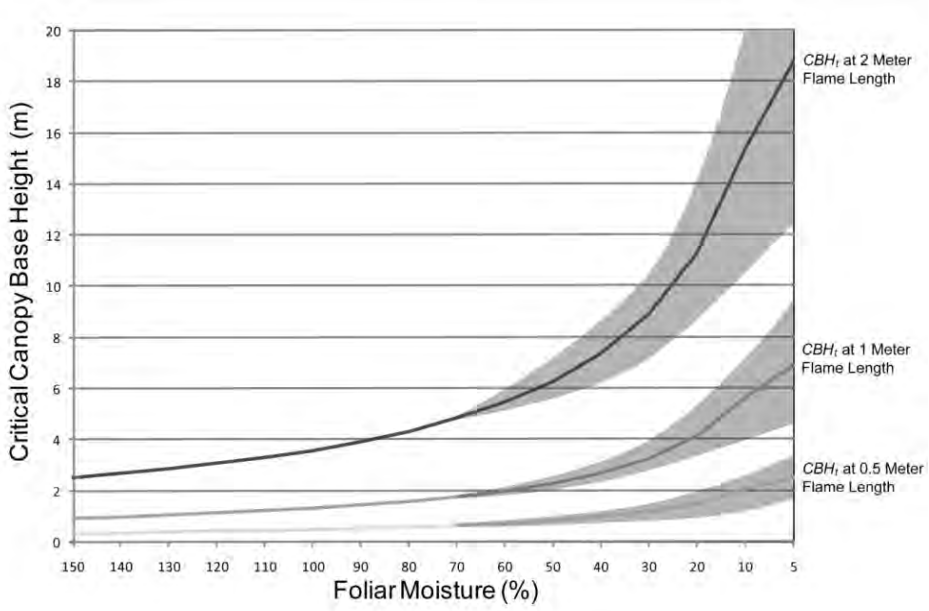

Figure 3—Critical crown base height (CBH_t) rises radically below a foliar moisture of 70 percent. The shaded area around the CBH_t less than 70 percent indicates a zone of assumed error due to lack of empirical data for these observed low FMC values.

10-Hour RAWS Fuel Comparison

A strong relationship appears to exist between RAWS 10-hour fuel moisture and daily FMC_{dead}. Using a time period between 1600 to 2300 hours was highly correlated with FMC ($R = 0.88$, $P < 0.01$; fig. 4).

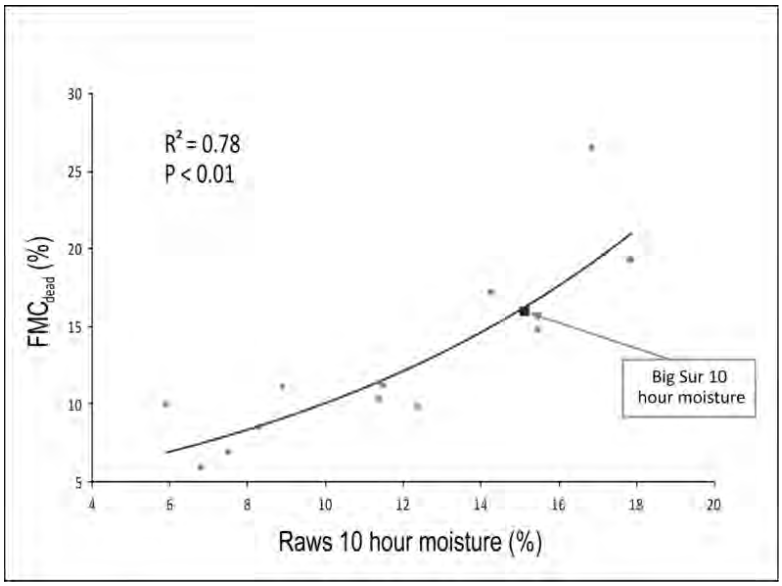

Figure 4—Relationship linking RAWS 10-hour moisture (average from 1600 to 2300 hours) and dead leaf FMC. The equation of the line shown is: ln Dead Leaf FMC percent = (1.3486) + (0.0955) RAWS 10-hour moisture percent.

Discussion

In this study, trees suffering from *P. ramorum* did not show a significant drop in FMC in the early infection stage. Throughout the sampling period, FMC remained almost unchanged, with ranges of ±2 percent in uninfected leaves and ±4 percent in infected leaves. The largest difference among the three infection categories was between the foliage of the dead trees and the leaves of uninfected/infected categories. Mean dead leaf FMC levels dropped as summer (fire season) progressed, allowing moisture to drop below 6 percent in August. Surface litter moisture beneath these same trees was only slightly higher than dead leaf FMC, averaging 7 percent moisture during the mid-summer months.

In current modeling of crown fire potential, the FMC range for most North American trees is between 75 percent and 250 percent. Values below 70 percent have not been reported. Extremely low FMC values, such as those found in the dead tanoak, exceed the range of data that generated Van Wagner's (1977) model. With FMC values approaching 5 percent, a "transition zone" is implied, since empirical data do not exist to verify this relationship. In this study we assumed a sequential error of ±5 percent for every 10 percent decrease in FMC below 70 percent. Future work will investigate the relationship of extremely low foliar moisture and CBH_t.

The relationship between RAWS 10-hour fuel moisture and FMC_{dead} is encouraging in that RAWS data may be a possible indicator of FMC of dead leaves. Due to the inherent lag of 10-hour fuel moisture, these values are capturing what has already transpired earlier in the day. This technique could be applied to evaluate an overall trend in foliar moisture pattern in dead tanoaks. More sample data are needed to verify the power of this relationship. It is interesting to note that samples take in the Basin Complex Fire (Big Sur, California, 2008) fit well on the prediction line (fig. 4).

On-going work will link increases in surface fuel loads generated by SOD (Moritz and others 2008; Valachovic and others, these proceedings) to coincident changes in canopy fuels. Results from this research will not only aid the decision support process for fire managers in SOD-affected areas, but may also be applicable to conditions in other ecosystems where diseases and insect epidemics have set up forests for future conflagrations.

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Interacting Disturbances: Did Sudden Oak Death Mortality in Big Sur Worsen the Impacts of the 2008 Basin Complex Wildfire?[1]

Margaret Metz,[2] Kerri Frangioso,[2] Ross Meentemeyer,[3] and David Rizzo[2]

Introduction

In late June 2008, a large, dry lightning storm ignited thousands of fires across California. The largest of these fires became the Basin-Indians Complex Fire in Big Sur, along the State's central coast. The fire burned over 240,000 acres (USDA Forest Service 2008) and required over a month of intense firefighting operations to contain the perimeter. Media reports and anecdotal accounts from firefighters linked the intensity of the fire and difficulty of firefighting operations to increased fuels from tree deaths caused by an emergent forest disease, sudden oak death (SOD).

Coastal California forests have experienced extensive mortality from the pathogen *Phytophthora ramorum*, causal agent of SOD (Rizzo and others 2005). The forests of Big Sur are among the most impacted by *P. ramorum*, with 100 percent of tanoaks in some stands infected by the pathogen and hundreds of thousands of dead host trees across the region (Maloney and others 2005). Big Sur is among the earliest sites of *P. ramorum* infection in California, and the pathogen has spread and become established throughout great portions of the region (Meentemeyer and others 2008).

We used an extensive network of forest monitoring plots in Big Sur to examine the potential interactions between these two important disturbance agents, a destructive exotic pathogen and wildfire. We used pre-fire data on tree mortality and pathogen distribution and post-fire surveys of burn severity to ask: i) How did pre-fire fuel loads vary among areas that differ in pathogen presence or impacts? and ii) Was burn severity higher in areas that had previously experienced higher SOD mortality? Ongoing research will track longer-term impacts of the fire on forest structure and recovery.

Materials and Methods

In 2006 and 2007, we established 280 intensive long-term monitoring plots across Big Sur as part of ongoing research to examine the changes in the forest community and environment that might result in positive or negative feedback between the

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pathogen, its various hosts, and the physical environment. The plots are randomly stratified among watersheds, in two forest types (redwood-tanoak or mixed evergreen), on public and private lands, and in areas with and without the presence of *P. ramorum*. In each 500 m^2 plot we quantified disease incidence, levels of tree mortality, amount of coarse woody debris, and various other biological and physical characteristics of the forest. Ninety eight of these monitoring plots were within the perimeter of the 2008 Basin Complex fire.

We used measures of standing dead basal area and downed log volume for both host and non-host tree species to understand pre-fire fuel loads in the region. Every standing dead stem ≥ 1 cm dbh (diameter at breast height, 1.3 m) was identified to species, measured for dbh, and classified according to an estimated time since death based on the presence or absence of fine fuels (leaves and fine twigs) or the fracturing and falling of major canopy branches. Downed logs ≥ 20 cm in diameter were identified to species and measured in 0.5 m length increments and 5 cm width increments to obtain a cylindrical volume.

In September and October, 2008, following containment of the Basin Complex Fire, we conducted a rapid response survey of 61 monitoring plots to assess burn severity using characteristics likely to disappear with the onset of California's winter rains. These included 30 mixed evergreen plots (nine uninfested, 21 infested) and 31 redwood-tanoak plots (10 uninfested, 21 infested). We rated plot-level burn severity using the Composite Burn Index (CBI), a rating from zero to three of the damage to several forest strata (soil, herbs, shrubs, intermediate trees, and dominant trees). We also took quantitative measures of soil and tree damage at eight random locations in each plot. We measured soil damage by assessing the depths of deposited ash, consumed litter and duff, and destroyed soil. We also measured the height of bole charring and canopy scorching or torching on the tree nearest the soil sample point.

We compared pre-fire fuel loads (standing dead basal area or downed log volume) from the full network of plots for lethal host species (tanoak, *Lithocarpus densiflorus*; coast live oak, *Quercus agrifolia*; and Shreve's oak, *Q. parvula* var. *shrevei*) using one-sided Mann-Whitney U tests, hypothesizing that host fuel loads were higher in plots where *P. ramorum* was present. Similarly, we compared burn severity in each forest type between infested and uninfested plots, hypothesizing that burn severity was worse where the disease was present. We used linear regressions of burn severity against fuel abundance (for lethal host species alone or all species) across both forest types and disease presence/absence to examine whether increasing fuels increased burn severity. We also separately analyzed the relationship in the 27 plots that contained recent SOD mortality and compared the relationship to that found in the 15 infested plots with older mortality. All our measures of burn severity were highly correlated, so we used the CBI as the outcome variable in the analyses presented here.

Results

Host fuels were significantly higher in plots with *P. ramorum* than in plots without the pathogen, as measured by host standing dead basal area and the volume of downed host logs. Despite great differences in host mortality, burn severity (CBI) did not differ between infested and uninfested plots in either forest type. The CBI also

showed no relationship with the amount of standing dead host basal area across all 61 plots.

We did observe the hypothesized increase in burn severity with increasing fuel loads when we examined the different types of fuels that occur over time as the pathogen becomes established in an area. Burn damage to the soil layer (as represented by the soil stratum component of the overall CBI) significantly increased with an increase in the volume of host logs. In plots with recent SOD mortality, where dead trees still possessed leaves and fine twigs when surveyed in 2006 and 2007, standing dead basal area (of all species) was a significant predictor of the overall burn severity (CBI), such that greater mortality led to increased burn severity across both forest types. In infested plots where mortality was older, and the trees had lost their fine fuels, there was no such relationship, however.

Discussion

The Basin Fire provided a unique opportunity to examine the potential for *P. ramorum* to have cascading effects on forest communities through interactions with wildfire. We hypothesized that SOD mortality would increase fire severity, but our results demonstrate that the relationship is complex, with great variability in SOD impacts and in burn severity across the region. Although host mortality increased significantly when the pathogen was present, burn severity showed little relationship with pathogen presence. We found that increasing fuel abundance did predict increasing burn severity when fuels were examined separately by type, however.

The effects of SOD on forest structure develop over several years as *P. ramorum* is dispersed to a new area, becomes established and begins to kill trees (Rizzo and others 2005, McPherson and others 2005). The quantity and quality of available fuel will correspondingly vary. For example, recently dead host trees may retain their leaves and fine branches for a year or more, resulting in a canopy full of very dry and highly flammable fuels. With time, these fine fuels will fall to the ground and decompose, and the larger branches in the canopy will also begin to fragment and fall. This will result in greater surface fuels in an area, and the rate of decomposition of these fuels will vary among species and habitat conditions.

Our results indicate that the timing of the fire in regard to the progression of the disease is an important predictor of burn severity because differences among fuel types were more important indicators of damage than pathogen presence alone. We found increased soil damage in plots with greater volumes of large downed logs. In higher severity plots, the fire consumed more of the litter or duff and destroyed soil to greater depths. The heat transfer to the soil causing this damage may also kill tree fine roots, and the soil damage will likely affect water flow and soil erosion throughout the plot. We also found that the abundance of standing dead biomass predicted burn severity only in areas that had recent SOD mortality, likely due to the presence of dry, fine canopy fuels, whereas no such relationship existed in plots where the pathogen had been established for longer periods of time.

Although there was an important and detectable relationship between SOD and fire severity, there remains much unexplained variation in fire severity. Fire behavior and spread depends on fuel availability, habitat characteristics and the climatic conditions

occurring on the day of the fire (Rothermel 1983). Big Sur contains a complex mosaic of habitat types, has steep topography, and has large temperature and moisture gradients. Mortality from SOD across the landscape varies by region and forest type (Maloney and others 2005, Meentemeyer and others 2008). The processes that determined fire severity during the Basin Complex fire likely occurred at multiple scales and were dependent on many factors, only one of which is the SOD mortality measured at the scale of our plots.

There are very few examples in the literature of post-fire ecological effects that are based on both pre- and post-fire data; most studies are based solely on post-fire examination of the landscape with no previous data (Jenkins and others 2008). Even rarer is the opportunity to examine interactions between a large wildfire and a destructive biological invasion. Ongoing research will expand our surveys of burn severity to a greater number of study plots and track tree mortality, forest regeneration, and pathogen survivorship and spread.

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Understanding the Long-Term Fire Risks in Forests Affected by Sudden Oak Death[1]

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Abstract

It is assumed that large numbers of dead and down tanoak in forests infested by *Phytophthora ramorum* contribute to increased fire hazard risk and fuel loading. We studied the impact of *P. ramorum* infestation on surface fuel loading, potential fire hazard, and potential fire behavior in Douglas-fir- (*Pseudotsuga menziesii*) dominated forest stands with a significant tanoak (*Lithocarpus densiflorus*) component in Sonoma, Mendocino, Humboldt, and Del Norte Counties in northern coastal California. We also tested the feasibility of using stands in which tanoaks were treated with herbicides as a proxy for stands that have been heavily impacted by *P. ramorum* over a long time period, especially in areas where stands have not been impacted by the pathogen over the desired timeframe. In each county, plots were established to assess surface fuel loadings in both *P. ramorum*-infested and uninfested forest stands. Plots were stratified by (1) whether tanoaks were killed by *P. ramorum* or by herbicide (as a surrogate for areas or time frames that were not present) and (2) length of time the plot has been known to be infested or since herbicide application (in groups of approximately 2, 10, and 20 years). Within each plot, environmental information, disease information, canopy characteristics, and fuel loading as measured by the planar intercept method, including forest floor bulk density measurements, were obtained. Stands treated with herbicides 5 to 8 years ago had significantly more surface fuel than stands treated more recently or those infested with *P. ramorum* within the past 5 years. Additionally, fuel amounts were very similar between stands treated with herbicide recently and recently infested stands, suggesting that further study may establish the former condition as a study surrogate for the latter. Fuel amounts from this study fit a fuel model from the existing literature fairly well, information that could be of use for fire behavior analysts and fire ecologists.

Introduction

Public and land manager concern about interactions between *Phytophthora ramorum*-caused tree mortality and fire has been present for some time, driven by the perception that oak and tanoak mortality in coastal forests contributes to substantially increased fuel loading. Changes in fuel dynamics range from overstory changes, as trees die and retain dead leaves for some time, to changes in surface fuel amounts and arrangement, as trees break and drop to the forest floor. Most predictions of fire spread and behavior on the ground are based on Rothermel's (1972) model that uses the number of surface fuel particles in several size classes as inputs along with several other measurable forest floor parameters; most predictions of crown fire

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ignition and spread are based on the model presented by van Wagner (1977). Relatively little work has been done to understand the nature and quantity of these fuels in areas infested by *P. ramorum*.

To address this perception, we studied the impact of *P. ramorum* infestation on surface fuel loading in Douglas-fir- (*Pseudotsuga menziesii*) dominated forest stands with a significant tanoak (*Lithocarpus densiflorus*) component in Mendocino and Humboldt Counties in northern coastal California[6]. In these geographic areas it is not known how long tanoak surface fuels persist. As a result of *P. ramorum*-caused mortality, will fuel levels be elevated for 5, 10, 20, or more years? *P. ramorum* has not been established in California forests for long enough to address these issues. Observations suggest that forest stands in which tanoaks were treated with herbicides to prepare sites for optimal conifer growth appear very similar to some stands heavily impacted by *P. ramorum.* Based on this observation, we tested the feasibility of using stands in which tanoaks were treated with herbicides as a proxy for the kinds of stands present farther south along the coast that have been heavily impacted by *P. ramorum* over a long time period. It was hoped that the observed fuel values in the plots in this study would then inform the construction of benchmark safety values for a Workplace Safety Analysis for fire suppression activities in areas affected by *P. ramorum.*

Methods

In each county, plots were established to assess surface fuel loadings in both *P. ramorum*-infested and uninfested forest stands. Locations of plots are depicted in fig. 1. Plots were stratified by (1) whether tanoaks were killed by *P. ramorum* or by herbicide (as a surrogate for areas or time frames that were not present) and (2) length of time the plot has been known to be infested or since herbicide application. The length of time was divided into "short" (approximately 2 to 5 years) and "medium" (approximately 5 to 8 years). A total of 35 plots were visited. Stand variability was reduced by selecting stands on southerly aspects (upper third of the slope where possible) with relatively young Douglas-fir and an abundance of tanoak that would match up with timber-type fuel models rather than shrub-type fuel models.

[6] This study is part of a larger ongoing study that includes Sonoma and northern Humboldt Counties. Additionally, other, simultaneous studies seek to address the crown fire segment of wildland fire dynamics in these forests (Kuljian and Varner, these proceedings).

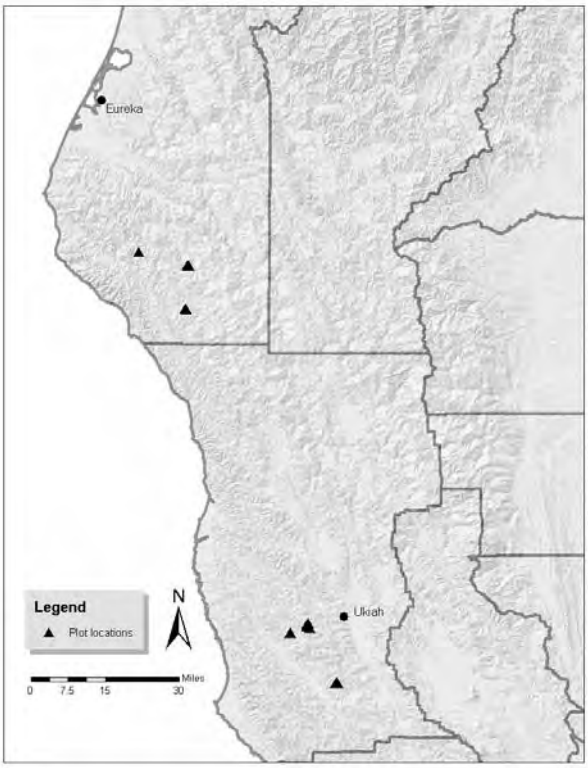

Figure 1—Plot locations in Humboldt and Mendocino Counties.

Plots were located randomly within the appropriate stands. Within stands infested by *P. ramorum*, plots were located at least 100 m from each other to avoid the likelihood that separate plots were part of the same infection event. Within each plot, topographic information (slope and aspect) was recorded, the UTM coordinates of the plot center noted, and observations made about the time since herbicide application or disease establishment, as well as a subjective measure of disease severity.

Stand information collected in each plot included (1) a variable-radius estimate of basal area obtained using a standard wedge prism; (2) an inventory of trees, both dead and living, within a fixed 1/10-acre (0.04 ha) circular plot, by species and diameter to the nearest inch; (3) presence or absence of foliage on each tree; (4) canopy position (dominant, co-dominant, intermediate, or suppressed) of each tree; and, (5) for selected trees, total tree height and height to the lowest live branch for the derivation of live crown ratios.

Surface fuel loading information collected included the standard variables measured for the planar intercept method described by Brown (1974). Three transects per plot were established at angles of 120 degrees from each other, with the first transect azimuth established randomly. The total length of each transect varied according to the distance to the nearest 1000-hour fuel particle (diameter >3 inches). Along each transect, data collected included numbers of 1-hour (diameter <1/4 inch), 10-hour (diameter ¼ to 1 inch), 100-hour (diameter 1 to 3 inches), and 1000-hour fuels and the condition of 1000-hour fuels (“sound” or one of four “rotten” categories), as well as duff and litter depths at three points along each transect. Additionally, forest floor bulk density was calculated at a random point within each plot by measuring duff and

litter depths at four points within a 30 cm X 30 cm square to calculate volume and then collecting and measuring dry weight of the litter and duff within the square.

Stand information was summarized. Surface fuel loadings between plots were compared using ANOVA. For each stand condition group, mean size class fuel loading by location was used to construct a custom fuel model and predicted fire behavior characteristics assessed using BehavePlus 4.0 (Andrews and others 2008), a fuel modeling and fire behavior simulator based on Rothermel (1972). Parameters for the fire behavior simulation described a typical warm, dry summer day (August 14 at 1400 hours, temperature 32.2 ºC, 22 percent relative humidity) on a south-facing 45 percent slope with surface wind speed from 0 to 8 km/hour (0 to 5 m/hour).

Results

Stand Conditions

Stand basal areas are summarized in fig. 2. As expected, the proportion of live tanoak decreased across a continuum of increasing time since treatment or infection—from control stands to *P. ramorum* early stands to herbicide medium (older) stands—and the proportion of dead tanoak increased, while the proportion of other species remained relatively constant. Additionally, total plot basal area decreased in the older plots because more killed tanoaks have fallen down. Regenerating Douglas-fir and other conifers in older plots were still generally small with the exception of occasional rapid-growing redwood stump sprouts.

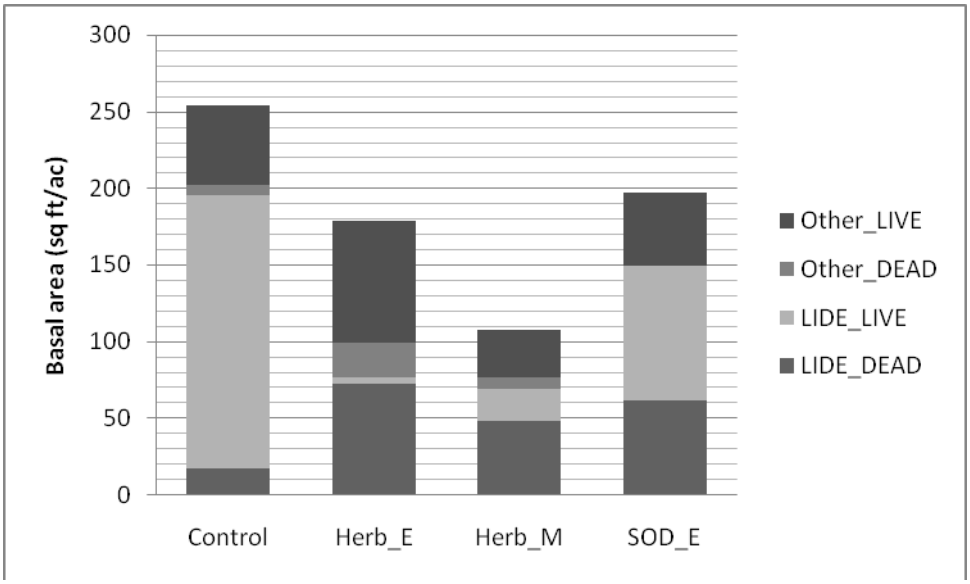

Figure 2—Comparison of stand basal area by species and stand condition type.

Distribution of trees by diameter class followed a standard reverse J-shaped distribution (fig. 3). *P. ramorum* early plots generally had fewer small trees than the other stand conditions, and the pathogen killed more codominant trees in these plots than those in other canopy positions (fig. 4). No dead trees in herbicide-killed or control plots, and relatively few trees in *P. ramorum*-affected plots, still had leaves

attached (fig. 5). The presence of dead, dry leaves in the canopy is a possible factor in crown fire risk (Van Wagner 1977). The presence of these leaves in *P. ramorum*-affected plots is probably related to the ongoing nature of tree mortality there, as opposed to the one-time pulse of mortality in herbicide-treated stands.

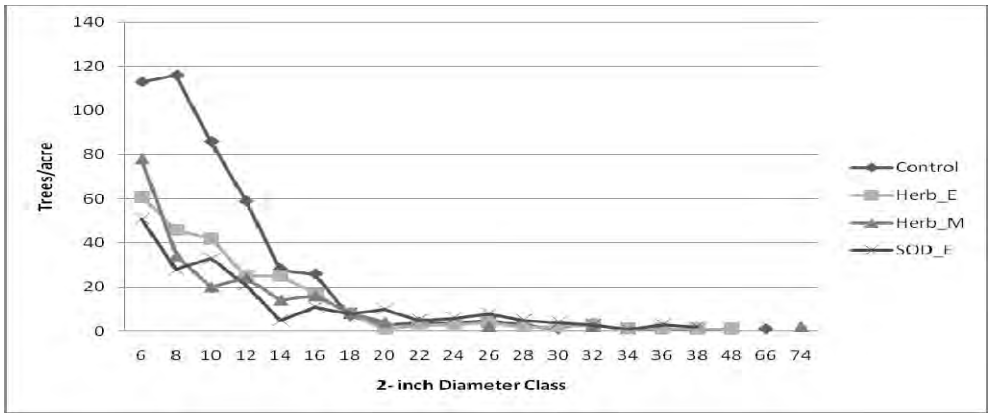

Figure 3—Number of trees by diameter class for each stand condition in the study.

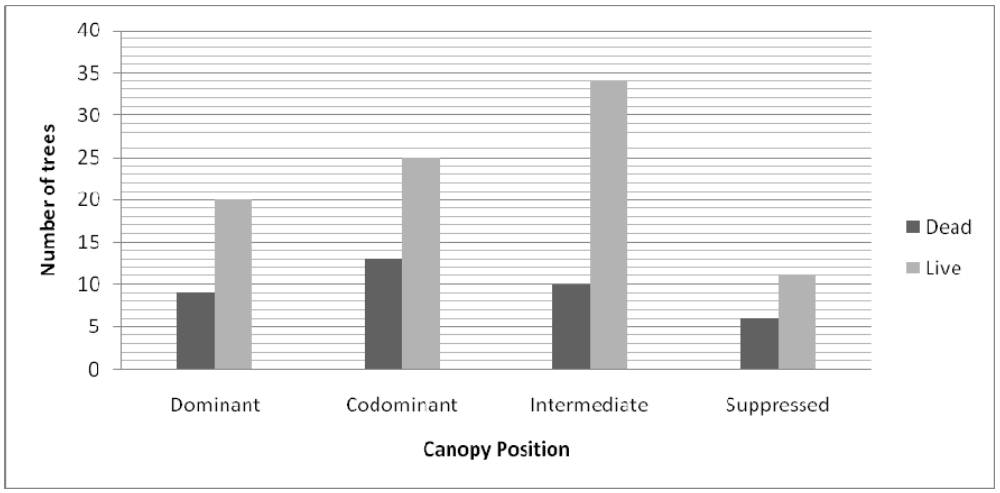

Figure 4—Living and dead trees by canopy position in *P. ramorum*-affected plots.

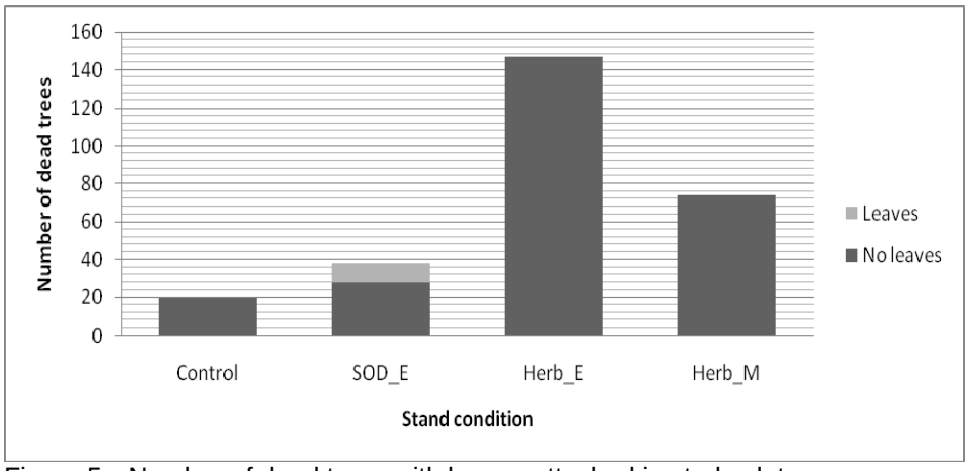

Figure 5—Number of dead trees with leaves attached in study plots.

Surface Fuel Loadings

Across all timelag fuel-size categories (1-hour, 10-hour, 100-hour, and 1000-hour), the herbicide medium stand condition had significantly more fuel than the other three conditions (control, *P. ramorum* early, and herbicide early). No significant differences appeared between these latter three treatments. The differences between the herbicide medium and other treatments are especially noteworthy (two to three times greater in the herbicide medium condition than the others) in the 1-, 10-, and 100-hour fuels (fig. 6). These are the most "fire-sensitive" fuels, in other words, the fuels that would contribute most to fireline intensity, flame length, and rates of spread (Agee 1993).

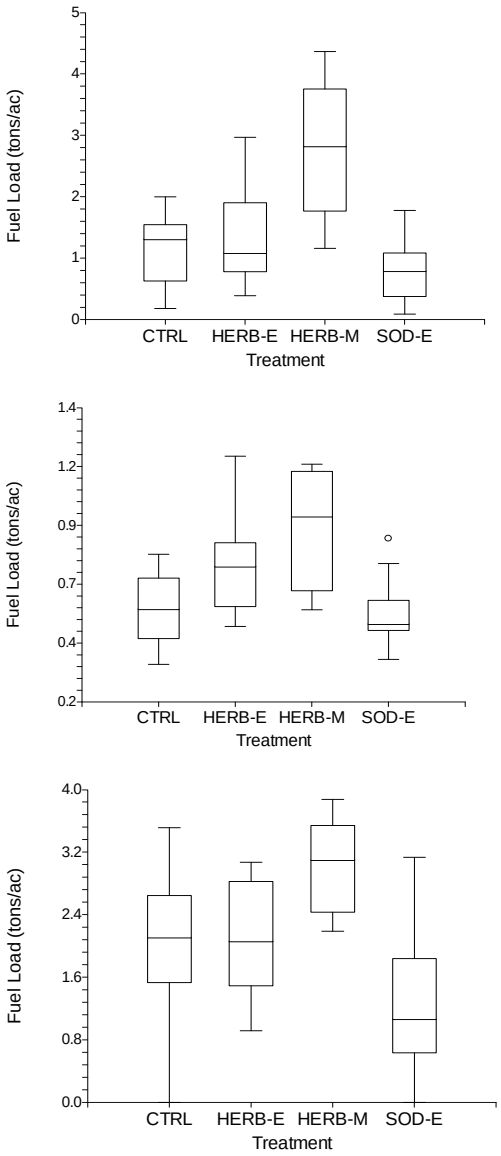

Figure 6—Comparison of surface fuel loading in tons/acre by stand condition. Top graph represents 1-hour fuels, middle graph 10-hour fuels, and bottom graph 100-hour fuels.

Phytophthora ramorum early and herbicide early conditions did not differ in 1-, 10-, 100-, or 1000-hour fuels or in litter depth. This provides some evidence that the herbicide early condition may be a relatively accurate surrogate for *P. ramorum*-infested stands during the first few years after infection, although further data collection is needed.

Fire Behavior

The fire models from the existing literature that most closely tracked the fire behavior predicted by the simulation were Model 8: Closed Timber Litter (Anderson 1982) and TL2: Low Load Broadleaf (Scott and Burgan 2005). Of the two, Model TL2 was superior. Whereas Model 8 over-predicted fire behavior produced from the fuel beds as sampled, Model TL2 was very close to the predicted values. Fig. 7 shows this for rates of fire spread. Predicted rates of spread matched the TL2 model somewhat better than did fireline intensity or flame length. In this figure, curves that continue to increase across the entire graph represent fuel models 8 and TL2; graphs that level off represent modeled rates of spread for the various represented stand conditions (the points of level-off represent wind speeds above which rates of spread do not continue to increase).

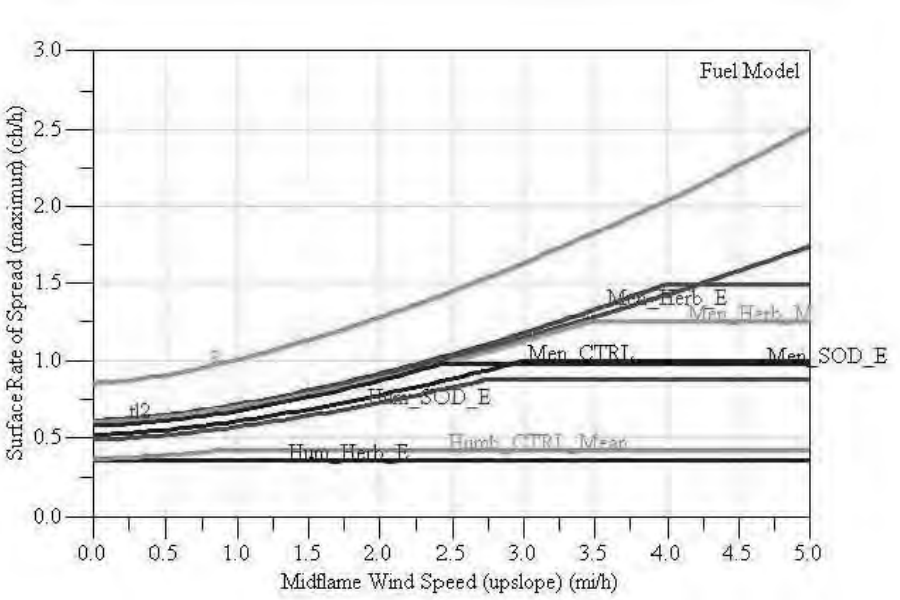

Figure 7—BehavePlus predicted rates of fire spread for stand conditions (separated by county) and for standard fuel model forest types according to Anderson (1982) ("8," complete curve at top) and Scott and Burgan (2005) ("tl2," other complete curve).

Discussion

Perhaps the most significant result of our study, and the one most useful to actual fire managers, is the discovery that, based on the data gathered so far, Model TL2 is a good fit for surface fuel loading conditions in *P. ramorum*- and herbicide-affected stands in Mendocino and Humboldt Counties. Land managers and scientists studying *P. ramorum* have questioned whether existing fuel models fit a situation with the unprecedented levels of tanoak mortality that are caused by this disease, or whether

the development of a custom fuel model might be warranted. This question is important because fire behavior analysts need to know which fuel models best approximate the conditions they see on the ground in a given area at the beginning of a fire incident. This helps them provide the most accurate fire behavior predictions possible in support of tactical and resource deployment decisions. Additionally, having an accurate extant model can help researchers who seek to understand the potential impacts of *P. ramorum* as they try to characterize those impacts for social, ecological, and economic analyses.

Since Model TL2 is a reasonably good fit, the creation of a formal custom fuel model for large amounts of hardwood mortality is probably not warranted at least for the two counties studied. The results suggest the possibility that the input of even large amounts of hardwood mortality to surface fuels, whether controlled by decay rates over time or by some other factor, is gradual enough—and the spatial arrangement of those fuels sufficiently dispersed over the area of mortality—that unusually large amounts of surface fuels do not build up at any one time.

This conclusion is provisional, however, for two important reasons. First, it includes only 2 out of 14 California counties infested by *P. ramorum*. Fire ecology can differ dramatically between different California ecoregions, even exclusively coastal ones (Sugihara and others 2006). Surface fuel accumulations might be expected to differ in redwood forests, or in mixed-evergreen forests dominated by hardwoods in the central coast. Second, the study does not yet include data on areas that have suffered severe hardwood mortality over time horizons longer than 5 years. It may be that tree failure rates and fuel accumulations cause more surface fuel accumulation at some point in time beyond 5 years.

Future study will go some way toward remedying these deficiencies. Study in Sonoma County (within which *P. ramorum* has been killing tanoaks for longer) and in the northern Humboldt County areas are scheduled soon, and further study will concentrate on plots that were herbicide-treated or where *P. ramorum* became established more than 8 years ago. These results also point out the need for further quantification of surface fuels in areas affected by *P. ramorum* in other parts of coastal California.

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Sudden Oak Death Mortality and Fire: Lessons from the Basin Complex[1]

Chris Lee,[2] Yana Valachovic,[2] Susan Frankel,[3] and Katie Palmieri[4]

Abstract

Land managers, fire suppression professionals, and research scientists have speculated about the relationship between increased *Phytophthora ramorum*-caused hardwood mortality and wildfire incidence, severity, and behavior in coastal California. Little quantitative data has emerged to measure the nature of any such relationship. The Basin Complex and Chalk fires in the summer and fall of 2008 along the Big Sur Coast provided the first opportunity for observers to confirm or disconfirm speculations about fire and *P. ramorum.* In an effort to focus research, outreach, and technical assistance, we conducted an information-gathering survey targeted at select personnel who worked on the Basin Complex and Chalk fires, and followed the survey with a series of meetings with land management professionals and scientists to obtain recommendations for how these firefighters' experiences should inform future research and outreach efforts. Recommendations included more effective provision of needed maps and safety information; future research into the best methods for sanitizing water or ensuring that infested stream water is not used to fight fire; investigation into characteristics of live fuels in areas of increased hardwood mortality to aid fire behavior analysts with predictions; and increased coordination with firefighting agencies for information distribution and standardization of demobilization procedures.

Introduction

Concern about potential interactions between fire and large numbers of dead hardwood trees killed by *Phytophthora ramorum,* the cause of sudden oak death (SOD), has been present for some time among scientists, land managers, and residents of the coastal California wildland-urban interface. It is unknown whether the amounts and configurations of fuels contributed to coastal forests by *P. ramorum* might make fires in those forests behave differently, might make them more difficult to suppress, or might contribute to a large-scale change in fire ecology. Most of the limited experience with, and research on, *P. ramorum* and fire has so far concentrated on single field-based (K. Julin, personal communication) or lab-based (K. Fischer, personal communication) case studies, on GIS-based analyses (Moritz and Odion 2005), or on speculation from land managers and the public on fire's probable effects on the pathogen. One study did compare ground surface fuel loadings in infested and uninfested stands in two forest types (redwood/tanoak and Douglas-fir/tanoak) at Point Reyes National Seashore, finding that fuel loadings were significantly greater in diseased redwood/tanoak forests than in Douglas-fir/tanoak forests, with most of this increased loading coming from the 1000-hour (>3 inches in diameter) fuel class. On the other hand, surface fuel loading was significantly greater in healthy Douglas-

[1] A version of this paper was presented at the Fourth Sudden Oak Death Science Symposium, June 15-18, 2009, Santa Cruz, California.

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fir/tanoak stands than in diseased Douglas-fir/tanoak stands, with most of this increased loading coming from greater amounts of duff (Moritz and others 2008).

The Basin Complex fire in Big Sur, which began on June 21, 2008 as several smaller fires that subsequently coalesced, burned 162,818 acres before being declared contained on July 27 (InciWeb 2008a). The western and northern flanks of this fire burned in several areas noted for heavy tanoak (*Lithocarpus densiflorus*) and coast live oak (*Quercus agrifolia*) mortality caused by *P. ramorum*. The Chalk Fire began later, on September 27, and burned 16,269 acres before being declared contained on October 30 (InciWeb 2008b). The Chalk Fire burned farther south along the coast than the Basin Complex, and like the former fire, it burned in areas containing trees killed by *P. ramorum.* This series of fires provided the first opportunity for interested observers to gauge the effects of increased hardwood mortality on firefighter safety, fire suppression operations, fire behavior, fire severity, vegetation response, and pathogen persistence in burned areas.

Methods

Systematic studies are underway to provide some of this information (D. Rizzo, personal communication; Metz, this volume). However, in an attempt to capture the perspective of experienced firefighters to inform a discussion of operational and information needs for people working on similar fires in the future, we launched a survey-driven effort that used a two-stage approach to consolidate the available anecdotal information. First, we administered a survey regarding fire behavior and fire operations in the Basin Complex and Chalk Fires to a core group of incident management team (IMT) personnel. Second, we convened a series of three web meetings with a variety of firefighting administrators, ecologists, and land managers to bring some of the issues raised by survey respondents under the lens of scientific knowledge for development of a concise set of recommendations for policy, education, and operations related to fire and SOD.

We distributed our initial survey to a small group of firefighting administrators with experience on the Basin Complex and Chalk Fires, obtaining additional contacts through iterative requests of the people whom we were talking to as well as through a posting on MyFireCommunity.net, a virtual community of wildfire professionals. As IMTs are composed of personnel from various agencies and states, we spoke to individuals from a variety of jurisdictions in both California and Oregon who worked on the fires. Since one of the longstanding questions about SOD and fire involved whether the creation of a custom fuel model is warranted for areas with heavy hardwood mortality, we made particular attempts to solicit participation from fire behavior analysts.

The survey addressed the following general topic areas:

Fire Behavior	Fire Operations
General changes in fire behavior	Maintaining fire lines
Spotting	Hazard trees/safety challenges
Rates of spread	Hot spots
Flame lengths	Mop-up
Energy release	Demobilization process/sanitation
Residence time	Water cleanliness
Difficulty in predicting fire behavior	Future research needs
Future research needs	

The core group of fire professionals who were administered the survey included 12 individuals with direct experience on the fires in Big Sur. Their responses informed the series of web meetings, which included 20 additional professionals. In each meeting, the general issues surrounding fire and SOD were presented as background material, and survey results were summarized for meeting participants. The first discussion specifically addressed fire operations issues, and the second addressed fire behavior and fire ecology. The third discussion synthesized the results of the first two discussions to reach conclusions on how best to move forward with integrated education and mapping efforts, policy recommendations, and future research needs.

Results and Discussion

Survey Results: Fire Behavior

Most striking about the responses from firefighting personnel who worked on the Basin Complex was the perception that *P. ramorum*-caused mortality had markedly increased surface fuel loading. This resulted in noticeably longer flame lengths, with one respondent commenting that quiet (~4-foot flame length) fires burning in grass fuel models sometimes increased to 20-foot flame lengths when they ran into areas of tanoak and coast live oak mortality. The same respondent estimated that the fuel loading has increased by a factor of five. Such fuel-fed increases in flame length can make direct attack tactics on the flame front infeasible and usually require firefighters to retreat to the nearest ridge to initiate back burning.

Indeed, this was reported numerous times during operations on the Basin Complex. Choice of tactics is not a value-neutral topic, as it can influence the eventual amount of forest burned (whether purposefully or not), structures chosen for protection or abandoned, and amount of involvement and input from the local community (Terence 2008, Sabalow 2008). One survey respondent explained that on the Basin Complex, community perceptions of the U.S. Department of Agriculture, Forest Service (USDA FS) management in the northern part of the fire, where the most tanoak and oak mortality was located, were more negative in general than perceptions of both the California Department of Forestry and Fire Protection (CAL FIRE) and the USDA FS management farther south in the Chalk Fire. This had to do at least partly with the amount of indirect attack necessary in the northern part of the fire.

However, it would be premature to conclude that increased hardwood mortality because of *P. ramorum* was solely responsible for repeated choices to use indirect

attack, since other respondents noted that changes in tactics are not uncommon in Big Sur, where rugged topography, fluctuating weather, and an abundance of fuels often cause increases in fire behavior that necessitate flexible decision making in regard to attack strategy. Also, areas of increased tanoak and oak surface fuels were patchy. One respondent noted that the fuels were arranged in "jackpots" and that the fire behavior increased and subsided cyclically as the fire traveled from jackpot to jackpot, while another noted that the Chalk Fire burned in such steep topography that most of the surface fuels had already rolled to the bottoms of draws before the fire started. Another respondent, however, observed a larger-than-normal amount of rolling flaming material on the northwest side of the Basin Complex, as fuels remaining on the tops of steep slopes ignited and fell down the slopes.

Additionally, most people who were surveyed noted greater-than-normal spotting activity, either through increased ember production or increased spotting distance, from standing dead oaks and tanoaks. Standing dead trees, especially during the period of time when dead, brown leaves remain on the tree, are both good generators of ember material and good receivers for windblown embers that begin new spot fires. Spotting distances were noted to be over half a mile in some cases. This is in contrast to the Indians Fire, which burned at the same time to the east and eventually merged with Basin Complex. The Indians Fire burned in areas with little tanoak and a relatively light pattern of coast live oak mortality. As a result, one survey recipient reported that spotting on that fire did not appreciably increase.

Short-range spotting also made fireline placement more difficult on the Basin Complex. Besides the difficulty of cutting firelines in areas with piled-up mortality, embers landing on the far side of the line and igniting new spot fires often invalidated efforts to construct new lines soon after they were made.

Compounding this problem, large numbers of *P. ramorum*-killed trees fell across firelines. In general, the hazard caused by large numbers of falling dead trees elicited the most unanimous concern of all the survey topics; as one firefighting administrator put it, "Everyone knows someone who has been hit by a tree, so it's the number one concern," especially because failure rates were very high on this fire. One respondent estimated that half the standing dead trees in burned areas fell over within a time window between 20 minutes and twelve hours of fire front passage—much more quickly than could normally be expected. Personnel could not easily tell which trees posed a hazard, as they fell in unpredictable patterns and at unpredictable times.

It was noted that the fires in the Basin Complex burned actively downhill even at night, when humidity decreased in areas above the inversion layer that was present. This is a common component of coastal California fire weather (Pyne and others 1996). As such, conditions that encourage active burning at night are likely to be present in other areas with accelerated hardwood mortality caused by *P. ramorum.*

The prediction procedures used by fire behavior analysts working on the Basin Complex were conventional. In the absence of specific data or knowledge about burning conditions in the forest type where the fire is actively burning, they fit their current visual observations to whichever of the 13 standard fuel models seems most applicable. This may also involve adjusting various parameters in fire prediction

modules such as FMA+[5] to fit observed deviations from the standard models. On the Basin Complex, analysts reported that the fire began in conditions that matched Fuel Model 2 (timber with grass understory, little to no surface fuel) and then transitioned to conditions that matched a modified version of either Fuel Model 11 (light logging slash) or Fuel Model 12 (medium logging slash). The differences in energy release and residence time between the latter two models are considerable, with the medium slash model generating much greater rates of spread, much more intense energy release and often firebrands (Anderson 1982), which can make control more difficult. However, both models differ from the Fuel Model 8 or 9 (timber with loose litter) that analysts might normally have expected to match the conditions in hardwood-dominated forests. Generally, fire behavior analysts expressed the opinion that the available fuel models, with sufficient experience on the part of the analyst in adjusting parameters to fit the individual situation, are sufficient to predict fire behavior in areas with heavy hardwood mortality. One respondent said that if the development of a custom fuel model could generate public interest and involvement in the issue, it might be worth the time and expense to develop it (see discussion below).

Survey Results: Fire Operations

To enhance fire suppression operations in areas of increased hardwood mortality, respondents recommended a number of measures that can be taken. Most of these measures involve information sharing. First on the list, as mentioned above, is the hazard of falling trees. Although firefighters receive daily briefings reminding them that trees killed by *P. ramorum* are likely to fail rapidly and unpredictably, they do not always know as they move about the landscape where they are in relation to areas of heavy mortality. In response to this need, respondents voiced their desire for reliable, fine-scale maps of mortality on the landscape to distribute to firefighters for safety purposes and also to use when making decisions about tactics and resource deployment. Some respondents also suggested that methods or technologies for rapid diagnosis of infected trees would also be very useful—a need that they share with others managing SOD.

The need for better and instantly available maps dovetails with a need for increased communication between fire behavior analysts working on different fires in scattered, far-flung coastal areas in California. According to one respondent, IMT members who observe unusual fire conditions on one incident are very likely to report these conditions to other IMT members within the same organization, but not necessarily to IMT members from other organizations. For example, communication about conditions in the field would flow freely from analyst to analyst within the Forest Service or within CAL FIRE, but not from analysts within the Forest Service to those from CAL FIRE or vice versa. Virtual communities like the one mentioned before in this paper are helpful, but not all fire management personnel are members of such communities. This spotlights a need for a central repository of information—both map information and informal communication—about fuels conditions throughout coastal California.

Another operations concern involved treatment of water used for firefighting. No unusual measures were apparently taken on the Basin Complex to sanitize drafted water to ensure that *P. ramorum* was not moved from infested streams to other areas

[5] Fuels Management Analyst Plus, a software package that predicts fire behavior, torching and crown fire activity, and tree mortality based on forest structure and fire weather information.

of the landscape. IMT members and fire behavior analysts typically had little opinion one way or the other about the need for this practice.

Opinions on the efficacy of sanitation procedures taken to decontaminate vehicles and equipment during the demobilization process varied widely. Some people believed that no out-of-the-ordinary procedures were implemented, while others cited a more extensive process involving water tank sterilization prior to leaving the area and the use of Lysol® for tool disinfection. One individual wondered what was done with the numerous rental vehicles used on the fires before they were returned. Respondents also had difficulty explaining where to obtain definitive answers to questions about the demobilization process. This testified to the decentralized nature of this part of fire suppression operations, in which each module made its own decisions regarding sanitation. However, another fire manager, who worked on fires in Mendocino County (another *P. ramorum*-infested county) during the same summer, mentioned that demobilization procedures are established at the unit level by CAL FIRE, which would indicate some level of standardization of the process. Follow-up contacts may be warranted on this issue.

Synthesis and Discussion

There was general agreement among respondents that increased hardwood mortality increased fire behavior (including rates of spread, fireline intensity, flame length, and spotting) in general—one fire behavior analyst opined that fire behavior generally increased by a factor of 20 to 25 percent. The mechanisms for this increase appeared to come primarily through (1) longer flame lengths and longer residence times because of increased surface fuel loads and (2) more unpredictable spread because of increased spotting related to dead leaves in the oaks and tanoaks.

However, respondents were split in their reactions to this increase in fire behavior. This split occurred between respondents who were local to the fire and those who traveled from out of the area to work on the fire. In general, local respondents were more united in their concern about the increase in fire behavior than out-of-area respondents. One form that this concern takes is a push for an increase in quantity and aggressiveness of fuels treatments. When asked about future research needs, several respondents were respectful of the need for more research, but said that fuel treatments should be an overriding priority for the money that might fund that research. Out-of-area respondents, however, seemed more inclined to say that while the Basin Complex “burned very well” and the fire behavior was sometimes extreme, they are always prepared for extreme fire behavior in this part of California—mortality or no mortality.

Among both groups, little support was voiced for the idea of developing a custom fuel model. It was suggested that effort should go instead into quantifying the nature of the live fuels that grow up through the dead fuels on the forest floor, as the number and arrangement of live fuels are the primary variable factors for adjustment in fire behavior prediction programs when utilizing slash-type fuel models.

In the ensuing discussions, deliberations of the foregoing issues among the larger group of scientists and land managers unearthed some solid directions for information-sharing and new avenues for exploring potential solutions for problems, while leaving some intractable issues more nebulous. With regard to fire operations, water treatment and mapping/information-sharing generated some definite

suggestions. One proposed option regarding water involved closing whole watercourses to water drafting, since individual water tender and engine operators do not have time or training to treat the water that goes into their tanks. This approach is taken by CAL FIRE in northwestern California to avoid moving water that is infested by *P. lateralis*, the cause of Port Orford-cedar root disease. However, it was noted that not all locations in coastal California have abundant water sources, so whatever water is present must be utilized; moreover, the likelihood of infecting trees or plants in the dry season in inland areas with water drafted from watercourses may be small. It was suggested that researchers investigate the efficacy against *P. ramorum* of new sanitizers and also the feasibility of using water drafting systems in which sanitizers are injected automatically into every tank of water, eliminating the need for direct handling of chemicals.

The USDA FS Forest Health Protection (FHP) performs aerial surveys each year that capture most of the detailed landscape-level hardwood mortality sought by firefighters. USDA FS FHP also has the capability of depicting the cumulative density of mortality in each mapped polygon (Z. Heath, FHP, personal communication). These maps, along with maps depicting infested watercourses, can be made available to firefighters through the Forest Resource and Assessment Program (FRAP) website maintained by CAL FIRE. Meeting participants mentioned that both CAL FIRE and USDA FS routinely use FRAP for data layer access during fire incidents. Regular updates to the mortality information will require a point person to serve as liaison between USDA FS FHP and FRAP personnel. Additionally, it was suggested that the availability of hardwood mortality and watercourse infestation maps should be advertised at California IMT and Safety Officer meetings. Other kinds of information, such as improved safety messages about snag hazards, can potentially be shared through the California IMT meetings as well.

The less tangible aspects of the synthesis conversations involved policy and public opinion. The availability, or lack thereof, of funding for fuels treatments in areas with high levels of hardwood mortality is a perennially difficult problem. It was noted that, while this issue was rated high on lists of issues eligible for American Recovery and Reinvestment Act funds, none of the proposed projects has yet received funding. Moreover, some of the counties with high levels of hardwood mortality do not have large areas of federally owned land, making it difficult for those counties to attract federal funding for fuels treatments. Another obstacle to timely implementation of treatments is documentation required for National Environmental Policy Act (NEPA) and California Environmental Quality Act (CEQA) requirements—documentation that must begin months or even years before projects can begin. Although streamlining these permitting processes in the name of environmental “emergencies” is an idea that is often proposed, in reality, authorities are rarely amenable to signing off on the kinds of programmatic commitments that would enable such projects to proceed at the levels needed.

Complicating the funding picture are the constantly varying nature of public opinion and constantly changing levels of awareness of fuels-related hazards on the landscape. One participant in the web meetings suggested that the public as a whole may actually be becoming **less** aware of fuel hazard issues in California. In this context, he suggested that although hardwood mortality does not present a new or unique problem—fuel is fuel—SOD provides a chance to capture the public’s imagination and harness energy for new fuels management efforts. As we look

toward the next fire season, finding a way to meet these thorny, unresolved challenges—as well as implementing the definite suggestions for action made at these meetings—is imperative.

Summary Recommendations

Following is a list of recommendations for action to improve firefighter safety, increase the effectiveness of fire suppression operations, and better understand fire behavior in areas with increased hardwood mortality.

- Coordinate mapping efforts between USDA FS FHP and FRAP to provide a *P. ramorum*-related mapping resource at two scales: mortality (including density within each mortality polygon) at a fine scale, and infested watercourses throughout the state.
- Investigate new sanitizers for water disinfestations.
- Investigate the costs and benefits of water drafting systems with injected sanitizers.
- Update the California Oak Mortality Task Force safety message for firefighters and investigate avenues for distribution.
- Initiate conversation with CAL FIRE units and USDA FS Pacific Southwest Region about improving demobilization procedures.
- Investigate the characterization of live fuels growing in areas of increased hardwood mortality for input to fire prediction programs.
- Provide these recommendations and the updated safety message to California IMT and Safety Officer meetings.

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The Social Impacts of Sudden Oak Death and Other Forest Diseases: A Panel Discussion[1]

Janice Alexander[2] and Chris Lee[3]

Abstract

This panel aimed to discuss the intersection of biology and society; specifically, how we balance competing social and biological concerns in regards to forest pests and land management in general. Four panelists began the discussion: Janice Alexander, Sudden Oak Death Outreach Coordinator for the University of California (U.C.) Cooperative Extension, Marin County and the California Oak Mortality Task Force; Dr. Lynn Huntsinger, rangeland ecologist, U.C. Berkeley; Chris Lee, Sudden Oak Death Coordinator for U.C. Cooperative Extension, Humboldt and Del Norte Counties; and Chuck Striplen, Research Associate with the San Francisco Estuary Institute's Historical Ecology program, a Native American, and a Ph.D. student at U.C. Berkeley. Members of the audience, which included researchers, land managers, and regulatory professionals, also presented their perspectives as the discussion progressed.

Some of the topics the panel considered and discussed with the audience are listed here: individual rights/needs vs. rights/needs of the community; respecting and learning from traditional values, uses, and management; resolving disputes among neighbors, including public and private landowners and users; managing across property boundaries; environmental justice – who benefits and who loses from various policies, whose rights/needs/values are recognized and whose are not; how to cope with absentee owners; incentives versus regulations; identifying incentives that would work; how to make decisions when there are tradeoffs; gaps in jurisdictions, unclear jurisdiction; appropriate public communication; public involvement (who, when, how); and how cultural and economic values of the resource are affected.

[1] A version of this paper was presented at the Fourth Sudden Oak Death Science Symposium, June 15-18, 2009, Santa Cruz, California.

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Posters

Status of California's *Phytophthora ramorum* Stream Survey[1]

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

Abstract

Since 2004, we have annually monitored *Phytophthora ramorum* in streams, creeks, and rivers throughout infested and at-risk areas of California by baiting with rhododendron leaves. This survey helps delineate the pathogen's range and can quickly detect its spread. Our sites are dispersed from Del Norte to San Luis Obispo counties on the coast, and in Butte, El Dorado, Nevada, Placer, and Yuba Counties of the Sierra Nevada foothills. Monitoring runs from February through June, the season of mild and moist conditions and thus highest pathogen activity.

We deploy rhododendron leaf baits of mature cuticle encased in fiberglass mesh bags into streams for 7 to 21 days, starting with the longest interval for the early cold season, and decreasing bait exposure time as the season warms and stream temperatures rise and cause leaves to degrade more quickly. Baits are deployed once per month from February through June. At the end of each monitoring period, bait leaves are retrieved, surface sterilized, and cultured on *Phytophthora*-selective media. *P. ramorum* and other *Phytophthora* species are identified visually using a microscope, and in certain cases, confirmed by DNA analysis of bait tissue or cultures.

New detections of *P. ramorum* in 2008 occurred primarily adjacent to previously known infestation centers such as Redway, Humboldt County, the Navarro River watershed, Mendocino County, and Big Sur in southern Monterey County. A new detection in the Little River at Van Damme State Park marked the northern-most report of the pathogen in Mendocino County at the time, and was the most distant case from a previously confirmed occurrence. No terrestrial inoculum source has yet been identified for this site or infested creeks in the city of McKinleyville, Humboldt County. To date in 2009, we have only detected the spread of *P. ramorum* westward from previously confirmed sites near Redway in Humboldt County while detection of the pathogen continued for most sites confirmed in previous monitoring years.

In 2008, the second relatively dry season in a row, the pathogen was cultured predominately from February and March samples. The most well-established *P. ramorum*-infested sites (for example, the Navarro River watershed) had the most consistent detection, while a few with previous detection were negative. In 2009, a season with more regular rainfall, the pathogen was detected at some sites into May, but nonetheless the total number of detections again declined sharply after March. This seasonal variation suggests that occurrence of *P. ramorum* in streams coincides with periods of pathogen activity, and that detection in waterways during dry periods may be diminished. Nonetheless, the pathogen's detection in both new and previously confirmed waterways indicate that this is a sensitive method for monitoring the spread of *P. ramorum*.

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[1] A version of this paper was presented at the Fourth Sudden Oak Death Science Symposium, June 15-18, 2009, Santa Cruz, California.

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Sampling Cankers and Lesions on Eastern Forest Plant Species for *Phytophthora* spp. and Associated Organisms[1]

Yilmaz Balci,[2] William L. MacDonald,[3] and Kurt Gottschalk[4]

Introduction

Our studies, begun in fall 2003 in eastern and central U.S. oak forests, have demonstrated that native, exotic, and new *Phytophthora* species are common to forest soils (Balci and others 2007, 2008). In sites where we have isolated *Phytophthora* from rhizosphere soil samples, collected around the base of oak trees, usually no aboveground cankers or lesions are found. However, some of the species found in our survey sites such as *P. cinnamomi*, *P. cambivora*, and *P. citricola* can cause bleeding cankers as well as foliar infections on species commonly found in eastern U.S. forest ecosystems (Erwin and Ribeiro 1996). Whether in *Phytophthora*-infested sites any of the cankers or lesions can be associated with *Phytophthora* remains uninvestigated. Thus we surveyed trees and understory plant species for any symptoms that resemble *Phytophthora* infection and performed isolations to detect and evaluate the incidence of this group of organisms. Other commonly associated organisms were also identified.

Material and Methods

Ten sites were chosen from the 2004 multi-state *Phytophthora* survey that proved positive for *Phytophthora* (Balci and others 2007). Sites with highest isolation frequencies and species diversity were selected. Each survey site consisted of a circular 50 m radius. At each study site, a survey of trees and shrubs was undertaken to examine stems for any bleeding or canker development. Such trees were examined for fresh expanding necroses by removing the outer bark layer. Several bark and attached wood samples from the outer edge of an expanding lesion were taken from each tree and placed in plastic bags. Before the isolation process, samples were washed several times to remove any oxidative staining and polyphenols. Small pieces of the necrotic inner bark tissues then were plated directly on a *Phytophthora* selective media (PARPNH) using half of the wood chips collected (Erwin and Ribeiro 1996). No surface disinfections were performed prior to plating of samples on selective media. A portion of the other half of the chips was plated on malt extract agar (MEA) containing 100 mg L^{-1} streptomycin sulfate to evaluate the

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most frequent organisms associated with canker formation. Surface sterilization prior to plating was as follows: wetting 1 minute in ethanol (96 percent), surface sterilizing for 5 minutes in sodium hypochlorite (4 percent), dipping for 30 seconds in ethanol (96 percent), rinsing in sterile deionized water, and drying on sterile filter paper.

At each site, leaves of understory plants also were surveyed for lesions that may represent *Phytophthora* infections. Suspect leaf samples were wrapped in paper towels in the forest and transferred in plastic bags to the lab. Sections of necrotic leaves were plated onto *Phytophthora* selective media (V8-PARPNH) as well as on malt extract agar after surface sterilization as described earlier.

Although isolations were primarily conducted from necrotic bark tissues, soil samples as well as roots were collected from around sample trees in order to evaluate the resident soils for the occurrence of *Phytophthora* species. In addition to the five oak trees sampled previously, five other tree species were sampled. Soil samples were tested for *Phytophthora* with an oak leaflet baiting technique as described in Balci and others 2007. Roots collected from each of the soil monoliths were washed with pressurized water, and any necrotic tissue processed similarly as the canker samples found on stems.

Results and Discussion

Some of the significant findings were as follows:

- Soil samples collected in 2006 yielded similar isolation results as in 2004 for *P. cinnamomi*. However, this was not true for *P. europaea* and *P. quercetorum*, suggesting fluctuation of population of different species over the years (table 1).
- In sites infested by *P. cinnamomi*, other tree species were also found to be infected by this species. In various West Virginia sites, beside the oak species, soils collected from the following plant species also resulted in positive isolation: *Acer rubrum*, *Aesculus octandra*, *Carya* sp., *Fagus grandifolia*, *Liriodendron tulipifera*, and *Nyssa sylvatica*.
- On coarse roots extracted from oak trees, multiple small lesions with a distinct margin were found. These lesions resulted only in few incidences with isolation of *P. cinnamomi* (table 1). The isolation success was very low and limited to one tree at a sampling site. In one site at Ohio, necrotic fine roots also resulted with *P. cinnamomi* isolation.
- Direct plating of root lesions on malt extract agar resulted with isolation of multiple fungal isolates. Among the isolates, two commonly isolated species, *Cryptosporiopsis* and *Cylindrocarpon*, are known to be pathogenic. Other potential pathogens include *Colletotrichum* cf. *trichellum* and *Cylindrocladium* sp.
- Lesions found on *Rhododendron maximum* and *Kalmia latifolia* were similar to *P. ramorum* infections found in nurseries. None of the necrotic leaves found on *Hamamelis virginiana* or *Magnolia* sp. resembled *Phytophthora* infection. In no instances was *Phytophthora* isolated from any of the foliar samples. Among the fungi isolated from leaves, *Pseudocercospora* sp. and *Pestalotiopsis* sp. were the most commonly isolated fungi on foliage of kalmia and rhododendron, respectively. These two species are known to be pathogenic on foliage of a variety of other plants.
- Most of the stem cankers with tarry exudates or bleedings could be classified as caused by insect damage, bacterial bleeding, frost, or wood-decay organisms.

However, in some incidences they closely resembled *Phytophthora* lesions. Despite the similarity of those lesions, no *Phytophthora* was isolated from any cankers in the study sites.

Table 1—*Phytophthora* spp. isolated from different plant parts and soil samples in 2006. All of the study sites were infested with a *Phytophthora* species, which were determined based on isolations from rhizosphere soil samples collected from oaks in 2004 (Balci and others, 2007)

State	Site	Soil		Root necrosis	Stem cankers	Leaf lesions
		2004	2006			
WV	Bakers Run Campgr	*P. cinnamomi*	*P. cinnamomi*	-	-	-
	Coonskin SP	*P. cinnamomi*	*P. cinnamomi*	-	-	-
	Beech Fork SP	*P. cinnamomi*	*P. cinnamomi*	*P. cinnamomi*	-	-
	Camp Creek SP	*P. cinnamomi, P. quercetorum*	*P. cinnamomi*	*P. cinnamomi*	-	-
	Chestnut Ridge Park	*P. cinnamomi*	*P. cinnamomi*	-	-	-
	Cacapon SP	*P. quercetorum*	-	-	-	-
PA	Buchanon SP I	*P. cinnamomi, P. cambivora, P. quercetorum*	*P. cinnamomi, P. cambivora*	-	-	-
	Buchanon SP II	*P. europaea, P. cambivora*	-	-	-	-
OH	Scito Trail SF I	*P. cinnamomi*	*P. cinnamomi*	*P. cinnamomi*	-	-
	Scito Trail SF II	*P. cinnamomi*	*P. cinnamomi*			

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An Evaluation of *Phytophthora ramorum* Preventative Treatments for Nursery and Forest Understory Plants in British Columbia, Canada[1]

Elisa Becker,[2] Grace Sumampong,[2] Simon F. Shamoun,[2] Marianne Elliott,[3] Aniko Varga,[4] Saad Masri,[4] Delano James,[4] Karen Bailey,[5] and Susan Boyetchko[5]

Abstract

Foliar treatments were applied to potted *Rhododendron* sp. cv 'Cunningham's white' plants over a 3-week period and evaluated for their ability to protect the leaves from *Phytophthora ramorum* infection. Treatments included several chemical and biological control products and systemic acquired resistance (SAR) elicitors. Excised leaves from treated plants were challenged by application of *P. ramorum* sporangia. Our preliminary results indicated that the most effective treatments were the two products registered for prevention of *P. ramorum* in Canada, Aliette® and Subdue Maxx®. Biocontrol treatments Rhapsody® and Actinovate® and SAR elicitors 3-aminobutyric acid (BABA) and Actigard® conferred a lesser degree of protection to the leaves and lowered the incidence of *P. ramorum* infection. Treatment with Sonata® was not significantly different than that with water. The chemical BABA did not inhibit *P. ramorum* growth *in vitro.* Plant trials are ongoing.

Introduction

On the west coast of British Columbia, Canada, several nurseries have reported *Phytophthora ramorum*-infected plants, but we have no indication whether the pathogen has spread beyond these points of entry. Plants in our forests and wildlands, as well as in our nursery industry and gardens, however, are vulnerable to this pathogen. As part of a collaborative research project among the Canadian Forest Service, Pacific Forestry Centre, the Canadian Food Inspection Agency and Agriculture and Agri-Food Canada (AAFC), we are evaluating treatments that may protect susceptible plants from infection, to be included in an integrated management approach.

Several commercially available biocontrol products, Rhapsody® and Sonata® (containing *Bacillus* spp.), and Actinovate® (*Streptomyces* sp.), were previously tested *in vitro* by using dual culture and treatments to detached leaves (Elliott and

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others 2008, in press). In the present study, we applied these treatments to whole plants and evaluated the response to *P. ramorum* infection on excised leaves of the treated plants. This plant trial also included treatments with chemical agents that may induce systemic acquired resistance (SAR) responses: Actigard® (benzothiodiazole), and 3-aminobutyric acid, (BABA) (table 1). We also included the chemical fungicides Subdue Maxx® (metalaxyl-m) and Aliette® (fosetyl-A), which are registered for use on *P. ramorum* host plants in Canada.

Methods

Plant Trials

Host plants were 1.5-year-old rooted cuttings of *Rhododendron catawbiense* cv. 'Cunningham's white' in gallon pots. Plant health was assessed before and after the trial. Eight foliar spray treatments were applied, as per manufacturers' label instructions or as noted for BABA (table 1), to five plants each. All treatments were applied once-a-week for 3 weeks, except for Aliette® and Subdue Maxx®, which were applied on a 2-week interval. Seven days after the last application of all treatments, 12 leaves per plant were excised and brought to the lab for challenge with *P. ramorum*. Sporangia were produced by growing *P. ramorum* isolate PFC 5073 (RHCC23), lineage NA2, in V8 broth for 4 days, then cultures were washed and media was replaced by water for 2 more days to induce sporangia formation. Cultures were poured through cheesecloth and the resulting purified sporangia solution was diluted to 10 000/ml. Half of the excised leaves from each treated plant (six) were sprayed with *P. ramorum* sporangia. All leaves were incubated for 14 days in sealed plastic boxes containing moist vermiculite, then disease response was assessed by counting the number of leaves with lesions as a percent of total leaves challenged with *P. ramorum* per treatment.

Table 1—Active ingredients and rates of foliar treatments to plants

Name, source	Active ingredient	Trial rate	PPM
Actinovate® SP, Natural Industries, Inc.	*Streptomyces lydicus* * added Silwet L-77	0.9g/litre	901ppm + 156ppm Silwet
Rhapsody® ASO, Agraquest	*Bacillus subtilis*	20ml/litre	20,000
Sonata® ASO, Agraquest	*Bacillus pumilus*	10ml/litre	10,000
Actigard®, Syngenta	benzothiadiazole	0.09g/litre	90.1
BABA, Aldrich	3-aminobutyric acid	1g/litre	1000
Aliette® WDG, Bayer	fosetyl-A	5g/litre	5006
SubdueMaxx®, Syngenta	metalaxyl-m	156µl/litre	156
Water			

Dose Response *In Vitro*

To evaluate the response of *P. ramorum* to BABA, 200µl cultures were initiated from 1000 zoospores per well in 96-well plates. Final concentrations of BABA ranged from 0.1 ppm to 1000 ppm. Plates were incubated at 20 °C. To estimate the growth of *P. ramorum* cultures, the optical density (OD) (650 nm) was measured every 24 hours for 3 days.

Results and Discussion

Our preliminary results of one plant trial indicated that the most effective foliar treatments for the protection of healthy rhododendrons from infection by *P. ramorum* were the two chemical fungicides registered for this use in Canada: Subdue Maxx® and Aliette® (fig. 1). Treatment of plants with the SAR elicitors BABA and Actigard® and biocontrol treatments Rhapsody® and Actinovate® resulted in some degree of protection to the leaves against *P. ramorum* and reduced the number of foliar lesions. Treatment effects of the biocontrol Sonata®, were not significantly different than treatment of plants with water (fig. 1). No treatments had any apparent phytotoxic effects. More assessments were made of this experiment than are presented here, including size of lesions and effects of leaf age. These data will be presented along with the results of the repeat of this experiment.

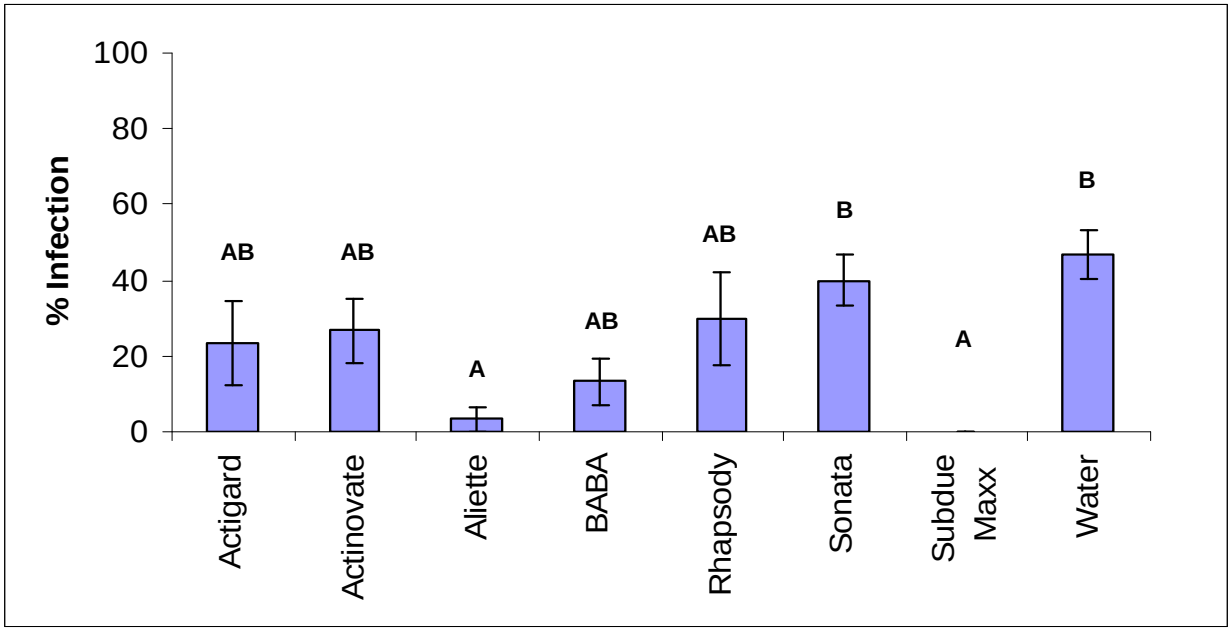

Figure 1—Disease response on excised leaves of treated plants. The number of leaves with lesions is given as a percent of total, per treatment. A one-way ANOVA comparing treatment means was performed. Treatments with the same letter were not significantly different according to the Tukey test at $P = 0.05$.

The dose response of *P. ramorum* to BABA was assessed *in vitro* over a wide range of concentrations. There was no significant difference in growth after 3 days among the cultures in different concentrations (0.01 to 1000 ppm) of BABA, showing equivalent results to growth in 0 ppm (P = 0.165) (data not shown). The chemical BABA did not

inhibit the growth of *P. ramorum* in liquid culture in concentrations up to 1000 ppm. This was the concentration used in foliar spray treatments (table 1).

More plant trials are underway on rhododendrons and other nursery and forest plants known to be hosts to *P. ramorum*. Root treatments are also being tested. One objective of these trials is to test whether resistance responses in treated whole plants can be detected in excised leaves challenged with *P. ramorum*. Infection of intact leaves on treated plants by *P. ramorum* will be performed in AAFC containment facilities and will be compared with the infection of excised leaves. Further *in vitro* screening is ongoing to identify other candidate treatments with suppressive effects against *P. ramorum* growth, including other microbials, plant extracts, and surfactants, as well as disinfectants for greenhouse use.

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Phytophthora ramorum Recovery from 12 Big Sur Watersheds Following Wildfires in 2008[1]

Maia M. Beh,[2] Kerri Frangioso,[2] Akiko Oguchi,[2] Kamyar Aram,[2] and David M. Rizzo[2]

Abstract

Many regions of California affected by sudden oak death (SOD) are prone to fire. Fire is an influential natural process in forest ecology, yet there have been very few studies on the effects of wildfire on forest pathogens, especially invasive pathogens. In the Big Sur area, where forests are among the most impacted by SOD, large areas were affected by fires during the summer of 2008, with over 240,000 acres burned in the lightning-sparked Basin-Indians Complex Fire and another 16,000 in the Chalk Fire. Field plots established in Big Sur in 2005 to examine the feedbacks between *Phytophthora ramorum* and the physical environment have provided information on pre-fire disease incidence, tree mortality, and various other biological and physical forest characteristics. Following the fires, burn severity, an evaluation of fire intensity, was also assessed using remotely-sensed as well as on-site plot measurements. Our pre- and post-fire data allow for the unique opportunity to study the interactions between SOD and wildfire. Some of the questions our lab is investigating include: Did SOD make the fires burn more intensely? Did the fires affect survival of *P. ramorum*, and if so, what biological and environmental factors were most influential in determining whether the pathogen survived the fires?

As part of our study examining the impact of wildfire on *P. ramorum* survival, we monitored 12 Big Sur watersheds for the pathogen in the spring of 2009. Stream monitoring, consisting of both baiting with rhododendron leaves and vacuum water filtration through 3 µM membranes, is a well-established method to assess *P. ramorum* presence on the landscape scale. Baiting provides information on cumulative pathogen presence over several weeks, while filtration allows for quantification of pathogen spores at a distinct time. Of the 12 watersheds, seven were burned in varying degrees and five were unburned. All of the watersheds except one (Big Creek) were known to contain *P. ramorum* before the wildfires. We were also interested to see if other *Phytophthora* species were present in different levels between burned and unburned watersheds.

Using the baiting technique, *P. ramorum* was recovered from five of the seven burned watersheds and four of the five unburned watersheds (Big Creek remained *P. ramorum*-free). There were no obvious temporal trends in *P. ramorum* abundance over the four bait deployment periods. From water filtration, over 250 isolates were subcultured and grouped into 25 morphotypes; only one isolate was identified as *P. ramorum*. Roughly half of the total isolates were suspected to be *Phytophthora* species, and these seemed to be equally distributed between burned and unburned watersheds. DNA sequencing of the ITS1-ITS4 region identified several of the filtration isolates as *P. gonapodyides* and *P. cactorum*. Future work includes additional DNA sequencing of water filtration isolates, as well as investigating *P. ramorum* survival in previously infested, burned terrestrial plots.

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Multiplex Real-Time PCR for Detection of *Phytophthora ramorum*, the Causal Agent of Sudden Oak Death[1]

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Abstract

Various molecular assays have been developed over the past few years for diagnostics of *Phytophthora ramorum*. The redundancy obtained by using multiple gene regions has increased the reliability in detecting the pathogen (Martin and others 2009). However, multi-gene assays require different PCR reactions to test a single sample. Having a reliable, sensitive, specific, fast, and low-cost molecular diagnostic assay is highly desirable for *P. ramorum*. In order to improve reliability and specificity without compromising speed and cost, we propose the development of two multiplex real-time PCR assays (Bilodeau and others 2009) using TaqMan probes with different reporter dyes targeting:
I) *Phytophthora. ramorum* and *Phytophthora* genus multiplex assay
Three different gene regions of *Phytophthora ramorum* (ITS, β-tubulin, elicitin) (Bilodeau and others 2007), *Phytophthora* genus (β-tubulin).
II) Hierarchical and RuBisCO multiplexing
Phytophthora ramorum (ITS), *Phytophthora* genus (β-tubulin), oomycetes (ribosomal 5.8S subunit) and host plants (*RuBisCO*), allowing simultaneous detection of *P. ramorum*, while verifying DNA extraction and the presence of other oomycetes in the DNA sample. The sensitivity of TaqMan assays in single and multiplex reactions was also compared in this study.

These assays were tested on different *Phytophthora* species and oomycetes, and were verified on two different sets of field samples previously assayed by other laboratories. These were obtained from multiple field hosts infected by various *Phytophthora* species and the DNA from one set was extracted from ELISA lysates.

When we used the multiplex assay combining three *P. ramorum*-specific TaqMan and one *Phytophthora* genus TaqMan, all samples containing *P. ramorum* were detected and no false negatives were obtained. However, some *Phytophthora* species cross-reacted with some probes. Nevertheless, in all cases, a false positive with one probe was accompanied by negative results for the other two *P. ramorum*-specific probes. This highlights the advantage of the redundancy generated in a multiplex reaction.

All *P. ramorum* samples from pure cultures or field samples were detected using these multiplex real-time PCR assays. In general, TaqMan multiplex assays showed lower detection sensitivity than single separate reactions and, in some cases, lower fluorescence. However, the

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multiplex assays still detected all *P. ramorum* accurately while decreasing the cost and increasing throughput.

The two multiplex assays developed here serve different purposes. The presence of an internal control (the plant TaqMan probe) should reduce the rate of false negatives by identifying reactions that have failed due to poor DNA extraction or to the presence of inhibitors. The different probes developed here should be useful for diagnostics of plant pathogens and have broader applications than just for *P. ramorum*. In plant health inspections or surveys, these probes could serve as detection tools. In particular, the *Phytophthora* spp., oomycetes and *RuBisCO* probes could be combined with other species probes for the detection of other plant pathogens. We expect that these multiplex PCR assays will be useful in the direct detection of *P. ramorum* from plant samples or ELISA solutions, as well as from cultures.

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Within Tree Intensive Sampling Leads to the Recovery of Multiple Genotypes of *Phytophthora ramorum* in Southern Oregon Tanoaks[1]

Jennifer Britt[2] and Everett Hansen[2]

Abstract

Phytophthora ramorum can infect leaves, stems, or the bark of plants; persists in streams and soils; and is even found in the xylem of oaks. Yet how *P. ramorum* infects and spreads through individual trees and spreads through forests is not completely understood. Individual trees often have multiple lesions on twigs in the crown, along branches, and on the main bole. Sometimes the lesions appear to be connected in the bark or wood and sometimes not. Several modes of infection are currently under investigation. These include mycelial spread within the tree, rain splash dispersal, and wind dispersal resulting in an initial infection in the tree canopy. In order to better understand how *P. ramorum* spreads through forests and individual trees or plants, we are using DNA fingerprinting (microsatellite markers) to track the movement of *P. ramorum* infections within trees in southern Oregon tanoak forests.

We isolated tissue from multiple crown and bole lesions of tanoak trees from 10 sites in southern Oregon. We collected samples from 2001 to 2007 in a variety of ways. For the within trees studies, trees were felled and the bark was scraped back to reveal lesions. In the crown studies, trees were felled and mostly stems were sampled. All other samples were collected during routine monitoring of southern Oregon forests by Oregon Department of Forestry personnel. Samples were plated onto *Phytophthora* selective CARP medium in the field and/or lab and grown out in the lab for identification (Hansen and others 2005). Isolates were genotyped at five microsatellite loci PrMS39, PrMS43, and PrMS45 (Prospero and others 2004), and PrM82 (Ivors and others 2006). Alleles were sized on an ABI Prism 3100 sequencer and results were analyzed using GeneScan and Genotyper software (Applied Biosystems). Approximately 80 percent of isolates were re-extracted and/or re-genotyped to confirm allele sizes.

A total of 54 trees from 2001 to 2007 were sampled at 10 sites in southern Oregon. Of those, 46 percent of tanoaks sampled had one genotype (25 trees) and 54 percent had greater then one genotype (29 trees), including one tree with eight different genotypes.

Mapping multilocus genotypes onto individual trees demonstrates multiple infections of *P. ramorum* by multiple individual zoospores in an individual plant are common. These results also provide insight into modes of *P. ramorum* infection. In about 25 percent of the trees with multiple genotypes there is vertical disconnection among genotypes where genotypes found at the top of the tree are different then at the base. This suggests the base of the tree is infected by rain splash versus the mid trunk which is likely infected by zoospores running down the trunk during heavy rains. Multiple genotypes found in the crown suggest a mode of infection

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via wind dispersal or rain splash by different *P. ramorum* individuals in the canopy. Once an infection is established, our results also suggest the pathogen can move through the wood via mycelial growth where one genotype is present and the lesions are connected. It is also possible that a single genotype from multiple individual zoospores can inflict multiple infections on an individual tree, where one genotype is dominant and clustered in three separate areas.

Alternatively, it is possible the microsatellites are mutating as they move through a tree. This would suggest the multilocus genotypes we found are not necessarily due to multiple infections, but are actually mutation in action. This is unlikely because many of the genotypes differ at more then one allele. Such a high rate of mutation has not, to our knowledge, been demonstrated in any organism. We are currently analyzing the results of a plating and sproutlet inoculation study where we genotyped individual isolates before inoculation and after growth to see if we can pick up a mutation signal in order to suggest the actual mutation rate of *P. ramorum*.

This work provides insight into how *P. ramorum* moves through individual plants post-infection and how an infection can move through a forest or nursery. It will hopefully help managers trying to track the spread of sudden oak death through a forest or across continents by molecular techniques. It also suggests that individual samples from a forest or nursery may not tell the complete migration story and care should be taken to collect a representative sample of isolates even within individual plants.

Acknowledgments

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Waiting for SOD: Sudden Oak Death and Redwood National and State Parks[1]

Monica Bueno,[2] Janelle Deshais,[2] and Leonel Arguello[2]

Background

Phytophthora ramorum is a highly aggressive, exotic pathogen responsible for the deaths of several million oak (*Quercus* spp.) and tanoak (*Lithocarpus densiflorus*) trees in California and Oregon and for leaf blight and twig die-back on over 100 other native and ornamental plants (Rizzo 2008). *Phytophthora. ramorum* and the disease it triggers, sudden oak death (SOD), has the potential to cause extensive ecosystem changes in the forests of Redwood National and State Parks (RNSP). The climate and forest systems of the north coast of California place RNSP in the highest risk category for SOD infection (Meentemeyer and others 2004). Although to date it has not been found in RNSP, the pathogen is present in forests 17 km north of the parks in Curry County, Oregon and in streams 15 km south of the parks in McKinleyville, California.

Redwood National and State Parks hold approximately 45 percent of the last protected old-growth redwood forests left in California as well as many thousands of acres of second growth forests. Tanoak is a major ecological component of these forests and is proving to be especially susceptible to *P. ramorum*. Tanoak populations infected by *P. ramorum* are approaching 100 percent mortality in some areas (Meentemeyer and others 2008, Moritz and others 2008) and the potential for local extinctions of tanoak is becoming more probable (Hansen 2008). Although the specific ecological consequences related to the loss of tanoaks in RNSP forests are unknown, we can assume there will be significant ecosystem changes due to the keystone/foundation species status of tanoak (Ellison and others 2005). In the forests of RNSP, tanoak is by far the principal mast producing species and provides foraging and nesting substrate for a variety of wildlife both on the tree itself and in leaf litter (Raphael 1986). RNSP also has an important cultural legacy of large stands of old tanoak trees that have been managed by Native American families for many generations.

Redwood National and State Parks has the unique opportunity to draw from over 10 years of SOD research and the management attempts of other agencies to develop a SOD management plan before the disease arrives in the park. It is the goal of the natural resources managers of RNSP to slow the inevitable arrival of *P. ramorum* to the parks by implementing preventive measures; to prepare for its arrival by conducting early detection for the pathogen and modeling the potential niche of its

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most important hosts; and to address the actions needed to confront the disease once it arrives by creating a SOD management plan based on ecological principles, the basic tenets of disease management, a strong knowledge of our forest systems, and early collaboration and consultation with regulatory agencies and adjacent land managers. It is our hope that through this process, the park can be prepared to act quickly and decisively to manage this pathogen when it finally does arrive.

Strategic planning

The first phase of our strategic plan consists of actions we are currently engaged in: prevention, early detection, and management planning. Our management plan will consider all tools available to combat this disease including strategies to eradicate, contain, conserve, and restore where possible (table 1). Our second phase will begin once the pathogen has arrived in the parks and we begin to implement the treatments outlined in the management plan.

Prevention

Our education and outreach program is geared toward informing RNSP staff and visitors about the seriousness of the disease and what they can do to help. We created best management practices brochures for employees, contractors, and researchers who work in RNSP and placed SOD information signs at visitor centers and trailheads. We also have a SOD link on the public National Park Service (NPS) website for RNSP.

Our prevention program also includes sanitation. We identified areas at high risk of exposure to SOD by human vectors, such as muddy areas near high densities of California bay laurels (*Umbellularia californica*) and tanoaks that are frequented by park visitors. In an attempt to reduce this potential risk we are graveling these areas to limit standing water and mud. This action is also helpful in our endeavor to reduce the spread of Port-Orford-cedar root disease caused by *Phytophthora lateralis*. Other prevention and sanitation measures, such as trail closures and cleaning stations, are being considered.

Early Detection

Early detection and a quick response are the keys to successful management of SOD. The RNSP early detection program includes stream baiting surveys, ground surveys, and aerial surveys (flown by the U.S. Department of Agriculture, Forest Service and ground checked by RNSP employees). We are also using an ecological niche model of host species to guide early detection efforts and direct management decisions once the disease has arrived.

Management Planning

Internal discussions are underway to understand the full array of options available to park staff to manage this disease once it arrives. Options being considered include: no action, preventive options, a containment strategy, an eradication strategy and forest restoration, in both the short- and long-term time frames. Important considerations in these discussions include funding, regulatory responsibilities (natural and cultural consultations), adjacent landowner actions and responsibilities, and public support. As part of our planning we may consider creating tanoak refuges

(defined as tanoak groves that are least likely to become infected due to spatial or temporal factors) and protecting them through the creation of no-host buffers. It is our goal that through this planning process RNSP will be able to more effectively and quickly implement the most appropriate treatment strategy at an initial infection site.

Sudden oak death management at RNSP will not be possible unless the treatment options are consistent with the overall goals for resource management at the parks and which take into consideration the high public profile position of the parks. The RNSP mission is, in part, to "preserve significant examples of the primeval coastal redwood forests and the prairies, streams, seashore, and woodlands with which they are associated..." as well as to maintain park forests "in a condition of unimpaired ecological integrity" (United States Department of the Interior 2000). We anticipate there will be much discussion and debate about how to best manage this disease and protect our forested ecosystems, consistent with park mission and values.

Table 1—Sudden Oak Death Strategic Planning Efforts at Redwood National and State Parks

Strategy	Action	Goal
	Current efforts (*P. ramorum* not present)	
Prevention	Education/Outreach BMPs for RNSP Information signs Public webpage Sanitation Gravel high-risk areas	To keep *P. ramorum* out of RNSP as long as possible.
Early Detection	Stream baiting Ground surveys Aerial surveys (USDA Forest Service) Host modeling	To find the pathogen before it becomes established in the landscape.
Management Planning	Communicate with regulatory agencies, adjacent land owners, and interested public on the full array of options available for managing this disease consistent with park values and authority.	To develop and implement a sound management strategy to combat this disease in the park.
	Future potential efforts after *P. ramorum* arrives in park	
Eradication	Set quarantine area Some combination of herbicide, cut, burn Monitor treated sites	To eliminate pathogen before it spreads to uninfected sites.

Protection/Containment	Chemical treatments AGRI-FOS®* – tanoaks Physical treatments Host removal in potential spread areas (buffers) Trail closures Cleaning stations	To strengthen important trees near infection sites and to prevent human and natural transmission of the pathogen.
Restoration/Conservation	Plant resistant hosts (if available) Plant conifers and other species Identify tanoak refuges and protect through physical and chemical buffers	To conserve ecologically important oaks and tanoaks in the landscape, rehabilitate infection sites, and minimize ecological impacts.

*Systemic fungicide approved for control and prevention of *P. ramorum* in California. AGRICHEM Mfg. Ind. Pty. Ltd.

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Effect of Fungicides on the Isolation of *Phytophthora ramorum* from Symptomatic and Asymptomatic Rhododendron Leaf Tissue[1]

Gary Chastagner,[2] Annie DeBauw,[2] and Kathy Riley[2]

Abstract

A number of systemic and contact fungicides, such as Subdue MAXX, Dithane, and Maneb, have been shown to be effective in controlling *Phytophthora ramorum* development on several nursery crops (Chastagner and others 2006, Garbelotto and others 2002, Heungens and others 2006, Linderman and Davis 2006, Tjosvold and others 2008). Studies on rhododendrons have also shown that some fungicides, such as Captan, have very limited residual activity, while residues of other fungicides, such as Segway, can significantly reduce disease development up to 92 days after application (Chastagner and others 2009). The use of fungicides to control *P. ramorum* has raised concerns that fungicide residues may affect the ability to isolate this pathogen from infected tissue, or more importantly, mask symptom development. Concealment of symptoms may make it more difficult to detect infected plants during routine visual inspections.

As part of our ongoing work relating to the management of *P. ramorum* on nursery stock, we conducted a study to determine if fungicide residues affected isolation of the pathogen from symptomatic tissue, and if any of the fungicides suppressed symptom development. Thirteen fungicides were included in this test (table 1).

The foliage on five container-grown 'Nova Zembla' rhododendron plants was sprayed with each fungicide during late August. Two days later, three leaves were removed from each plant and inoculated with suspensions of zoospores from an NA1 lineage rhododendron isolate of *P. ramorum*. A total of six 10 ul drops of suspension were pipetted onto the lower leaf surface, three on each side of the leaf midrib. The leaf tissue beneath three drops on one side of the leaf midrib was injured using an insect pin, while the tissue beneath the drops on the other side of the leaf was left unwounded. Checks included inoculated and non-inoculated leaves from untreated plants.

After 7 days incubation at 19 to 20 ºC, the leaves were photographed and the resulting leaf spots were measured using the APS ASSESS program. To evaluate the possibility of adverse fungicide effects on isolation of *P. ramorum* from symptomatic and asymptomatic inoculation sites, leaves were surface-sterilized in a 1:9 dilute bleach solution for 30 seconds prior to plating tissues from all of the inoculation sites onto CARP selective medium.

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Table 1—Fungicides Included in Test

Product	Active Ingredient
Captan 80 WP	captan
Disarm 480 SC (TM437)	fluoxastrobin
Dithane 75 DF	mancozeb
Gavel 75 DF	mancozeb + zoxamide
Heritage WG 50	azoxystrobin
Insignia 20W	pyraclostrobin
Maneb 75 DF	maneb
NOA 446510/A12946	mandipropamid
Polyram 80 DF	metiram
Ranman 400SC (Segway)	cyazofamid
Stature DM 50 WP	dimethomorph
Subdue MAXX FV	mefonaxam
V-10161 4FL	fluopicolide

Isolations from fungicide-treated leaves indicated that none of the fungicides tested had any adverse effect on the recovery of the *P. ramorum* from symptomatic tissue. *P. ramorum* was recovered from 98.9 percent of the 933 symptomatic inoculation sites. However, isolations from asymptomatic tissue suggest that Subdue MAXX and Insignia fungicides may pose a high risk of masking symptom development on rhododendron. *P. ramorum* was recovered from 12.1 percent of the 947 asymptomatic inoculation sites. About 67 percent of the asymptomatic inoculation sites that yielded *P. ramorum* were on leaves that had been treated with Subdue MAXX. About 17 percent were from leaves that had been treated with Insignia. If additional trials confirm these results on rhododendron and other hosts, growers could increase the effectiveness of visual inspections by using fungicides that do not adversely affect symptom development.

Acknowledgments

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Effect of Surface Sterilization Treatments on the Detection and Viability of *Phytophthora ramorum* on Various Substrates[1]

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

Abstract

An accurate evaluation of asymptomatic colonization of plant tissue by *Phytophthora ramorum* requires the ability to distinguish between the surface contamination or epiphytic growth of the pathogen and the colonization of plant tissues. Growth on a selective medium, such as CARP, following the surface sterilization of plant tissue is often used to confirm *P. ramorum* colonization of the tissue. Isolations and the use of PCR to detect asymptomatic colonization require that epiphytic pathogen propagules and residual pathogen DNA are rendered undetectable. A series of surface sterilization tests were performed with two commonly used laboratory surface sterilants to determine their efficacy in removing epiphytic propagules and DNA of *P. ramorum* in several different experimental scenarios. Substrates tested include detached rhododendron (*Rhododendron* sp.) leaves, rhododendron leaf discs, and freshly harvested Douglas-fir (*Pseudotsuga menziesii*) wood. Whatman filter paper was included to represent a non-infectable substrate.

Results indicate that the efficacy of a treatment varies by experimental scenario and detection method.

Rhododendron leaves and leaf discs: Based on post-sterilization growth on CARP medium, a 30-second treatment in a 10 percent solution of household bleach (0.6 percent sodium hypochlorite) 1 hour after a zoospore suspension of *P. ramorum* was applied to rhododendron leaves and leaf discs was as effective as higher concentrations of bleach or longer treatments in bleach in killing the pathogen on the surface of this host. A reduced effectiveness of sterilization treatments after a 3-hour post-inoculation incubation suggests that infection had already occurred by the time of treatment.

Filter paper: To investigate the ability of sterilization treatments to eliminate epiphytic propagules and residual pathogen DNA from the surface of a non-infectable substrate, discs of Whatman filter paper were inoculated with a *P. ramorum* zoospore suspension, treated with various sterilization treatments, and evaluated by quantitative PCR. Removal of pathogen DNA with a 30-second treatment with 10 percent household bleach was as effective as higher concentrations or longer exposure times. Neither 95 percent ethanol nor water was effective at removing detectable *P. ramorum* DNA.

Douglas-fir wood chips and filter paper: Douglas-fir wood chips and filter paper discs were inoculated with a *P. ramorum* zoospore suspension, incubated for 2 days, treated with various surface sterilants, and then evaluated for presence of viable *P. ramorum* (isolation) and DNA

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(qPCR). A 30-second treatment of 10 percent household bleach, 95 percent ethanol, or water was effective at eliminating detection of viable epiphytic *P. ramorum* on filter paper by isolation, but only the bleach treatment eliminated detection of *P. ramorum* DNA by qPCR. None of the treatments affected the detection of the pathogen by isolation or qPCR that had internally colonized the Douglas-fir wood chips.

Phosphonate Treatment to Control *Phytophthora cinnamomi* Infection of Ione Manzanita, *A. myrtifolia*[1]

Ellen Crocker[2] and Matteo Garbelotto[3]

Abstract

We are investigating the use of the phosphonate Agri-Fos® as a preventative treatment for *Phytophthora cinnamomi* infection of *Arctostaphylos myrtifolia*, Ione manzanita. *A. myrtifolia* is a rare, federally threatened species endemic to the acidic, iron-oxide clay soils of the Sierra Nevada foothills.

Habitat changes from mining, development, and changes in fire frequency have contributed to its decline, and in recent years *P. cinnamomi* root and crown rot has further increased mortality. *P. cinnamomi* is a widespread pathogen that attacks forest, ornamental, and agricultural plants, and while phosphonate is commonly used to control *P. cinnamomi* infections in general, its specific use for *A. myrtifolia* is unknown. The primary goal of our study is to develop a treatment regime for *A. myrtifolia*, first determining the optimal: 1) concentration of phosphonate/surfactant solution and 2) time of year for application.

To answer these questions we treated sample plots of *A. myrtifolia* with the following surfactant solutions in the fall and spring: 0.005x Agri-Fos® without surfactant, 0.025x Agri-Fos® without surfactant, 0.05x Agri-Fos® without surfactant, 0.005x Agri-Fos® with surfactant, and 0.025x Agri-Fos® with surfactant. At regular intervals cuttings were taken from each plot, as well as control non-sprayed cuttings, and inoculated with either *P. cinnamomi* (P3232) or V8 agar control. Eleven days after inoculation lesions were measured.

One month after application, both the spring and fall treatments significantly reduced lesion length in infected cuttings. All tested solutions, regardless of concentration and surfactant presence, appeared equally effective. Seven months after application, the spring treatment continued to significantly reduce lesion length in infected cuttings, but results from the fall treatment were mixed. For the spring treatment, all tested solutions were still equally effective; however, only four of the five fall treatments significantly reduced lesion length and even those appeared less effective than their spring counterparts.

These results suggest that spring Agri-Fos® treatment may be a useful tool in preventing *P. cinnamomi* infection of *A. myrtifolia*. Because all concentrations appeared equally effective, even a 0.005x topical application would reduce infection while having a minor environmental impact. While Agri-Fos® treatment seems promising, further studies are needed before any recommendations can be made. Because this treatment is somewhat phytotoxic, it is important to determine whether the benefit to the plants outweighs the damage caused. Future studies should determine whether Agri-Fos® is a good option despite phytotoxicity and how phytotoxicity can be reduced. Ideally, this improved understanding of treatments will enable us to outline a landscape level approach to prevent *P. cinnamomi* infection of *A. myrtifolia*.

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Predicting Sudden Oak Death in Redwood National and State Parks: Ecological Niche Modeling of Key Transmission Host Species Using Maximum Entropy[1]

Janelle Deshais,[2] John Stuart,[2] Steven Steinberg,[3] and Leonel Arguello[4]

Introduction

Phytophthora ramorum, the cause of sudden oak death (SOD), is an aggressive introduced plant pathogen that has caused the death of several million tanoak (*Lithocarpus densiflorus*) and oak (*Quercus* spp.) trees in the Pacific Northwest of the United States (Rizzo 2008). Primarily an airborne pathogen, *P. ramorum* spreads from its hosts via wind-driven rain. Not all hosts are equal; in the Pacific Northwest, tanoak and California bay laurel (*Umbellularia californica*) are most critical for wildland pathogen transmission (Rizzo 2005), and in the United Kingdom, rhododendron species (*Rhododendron* spp.) are spreading the pathogen (DEFRA, 2007).

To date, *P. ramorum* has not been detected in Redwood National and State Parks (RNSP); however, the pathogen is rapidly approaching. The Curry County, Oregon infestation is 17 km north of RNSP's Jedediah Smith State Park, and the recent detection of *P. ramorum* in Norton and Mill Creeks, McKinleyville (Humboldt County, California) is less than 15 km southwest of RNSP's southern border. Furthermore, key disease transmission species, California bay laurel, tanoak, and Pacific rhododendron (*R. macrophyllum*), are abundant throughout much of RNSP. Previous disease spread models predict RNSP in the highest risk category for SOD disease susceptibility (Meentemeyer and others 2004, Guo and others 2005, Kelly and others 2007). These spread models, however, predict establishment and risk on a scale larger than RNSP needs to aid management decisions. Also, vegetation data used in existing models are primarily derived from remotely sensed data which can have limited success deciphering understory species in old-growth redwood forests.

Our main objective in this study was to create detailed (≤ 30 m² resolution) species distribution maps for California bay laurel, tanoak, and Pacific rhododendron throughout RNSP. Once model inputs are refined to yield highest possible accuracy, we will use resulting species distributions to create a local-scale spread model. Final host distributions and our spread model will be used to understand *P. ramorum's* potential spread extent and lethality within RNPS's forests, as well as to guide management decisions in the event that *P. ramorum* does arrive.

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Methods

All species distribution modeling was carried out using MaxEnt v3.3, a niche-based modeling program (Phillips 2005, Phillips and others 2006). We chose MaxEnt based on its ability to handle categorical data as well as its use of presence-only records. MaxEnt uses deterministic algorithms to create probability distributions, with maximum entropy, for each target species based on a given set of predictor variables (Phillips and others 2006). The modeling process relates occurrence of a species to the broadest range of predictor variables, while optimizing predictive power.

MaxEnt requires species occurrence records and raster-formatted predictor variables. All occurrence records were compiled, *post hoc*, from 11 vegetation surveys that yielded a total of 1,606 unique point locations containing the presence of at least one of our target host species. We ran analysis with all available host presence points as well as with a subset that excluded points without confirmed geographic coordinates. There were a total of 1,188 occurrence records available for tanoak, 566 for Pacific rhododendron, and 216 for California bay laurel. The subset yielded 948 records for tanoak, 426 for Pacific rhododendron, and 187 for California bay laurel. For all analyses, 50 percent of occurrence records were used for model training and the remaining 50 percent were held aside for model validation.

We tested seven environmental data layers (table 1) in raster format, managed in ArcGis 9.3 (ESRI 2008). To minimize effects of autocorrelation within our models, we included bias files (using bias option in MaxEnt) that described relative sampling intensity (per survey area) throughout the study extent.

Table 1. Parameters tested in preliminary niche models predicting presence of *Umbellularia californica*, *Lithocarpus densiflorus*, and *Rhododendron macrophyllum* throughout Redwood National and State Parks.

Raster resolution
10m²
30m²
*Species records**
All available
Subset (excluded records without known GPS point)
Environmental data
Aspect (transformed to 0-180° scale, reclassified into 8 classes)
Degree slope
Distance from ocean
Elevation
Slope position (continuous percentage)
Slope position (5 classes)
Soil type
Vegetation type

*Different bias layer, depending on which set of species records used

For each species we tested eight unique parameter combinations. Each trial included aspect, degree slope, distance from ocean, elevation, soil type, and vegetation type; however, we also ran each model in 10 m² and 30 m² resolutions, with both sets of species records, and tested slope position in two different formats (table 1). We then chose the highest performing combination (based on largest area under curve (AUC) value – see below) and reran the model with 10 replicates (using random seed, with replacement). In all analyses we enabled the jackknifing option to assess relative importance of each predictor variable used in the model.

Results

Our preliminary results were encouraging, with all models performing significantly better than random [receiver operating characteristic (ROC) AUC values > 0.5] and p < 0.0001]. California bay laurel was best predicted by MaxEnt, followed by Pacific rhododendron and tanoak, with AUC values of 0.914, 0.743, and 0.701, respectively (table 2). Models with AUC values above 0.7 are generally considered useful, while values above 0.9 indicate high performance (Swets 1988, Elith and others 2006). Each species was modeled using slightly different sets of parameters (table 3). Final species presence/absence distributions were produced by converting the continuous logistic outputs (probability of presence) generated by MaxEnt, to binary outputs (presence/absence) using threshold values (fig. 1, table 2).

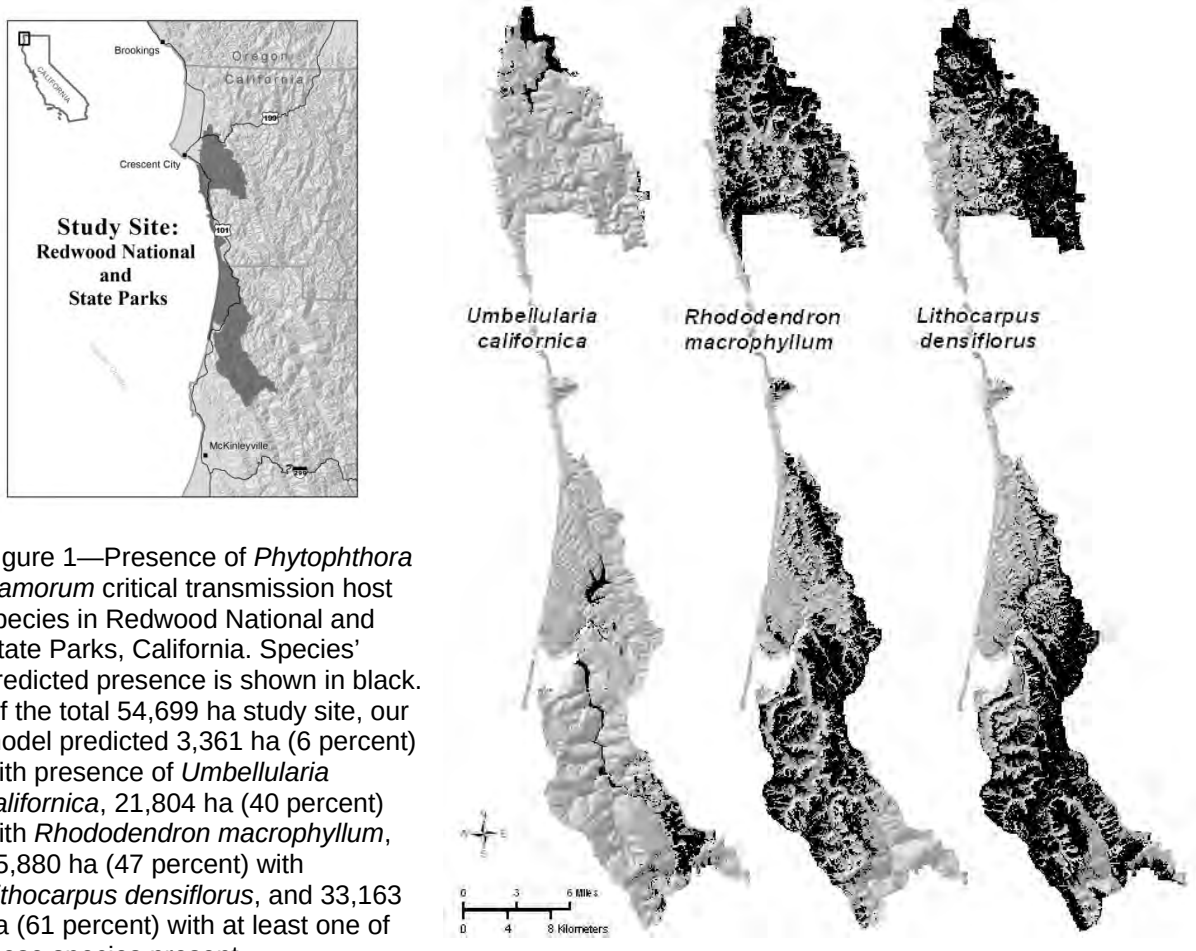

Figure 1—Presence of *Phytophthora rramorum* critical transmission host species in Redwood National and State Parks, California. Species' predicted presence is shown in black. Of the total 54,699 ha study site, our model predicted 3,361 ha (6 percent) with presence of *Umbellularia californica*, 21,804 ha (40 percent) with *Rhododendron macrophyllum*, 25,880 ha (47 percent) with *Lithocarpus densiflorus*, and 33,163 ha (61 percent) with at least one of these species present. Presence/absence predictions were calculated using binary thresholds listed in table 2. All modeling was carried out in MaxEnt v3.3 (Phillips and others 2006).

Table 2. Mean accuracy of preliminary niche models generated in MaxEnt v3.3. Each mean is calculated from ten replicates (with replacement) of the 'best' model design (largest AUC) for each species.

Species	AUC (STDV)	Sensitivity (STDV)	Binary Threshold	*p* Value
Umbellularia californica	0.914 (0.019)	0.907 (0.020)	0.388*	<0.0001
Rhododendron macrophyllum	0.743 (0.017)	0.763 (0.083)	0.317**	<0.0001
Lithocarpus densiflorus	0.701 (0.011)	0.862 (0.056)	0.324**	<0.0001

*Logistic threshold value set where model sensitivity (true positives) equals specificity (false positives)
**Logistic threshold value set to maximize sensitivity plus specificity

Table 3. Relative mean contribution of the four most informative environmental variables used in each model. All means were calculated from ten replicates (with replacement) of the 'best' model (largest AUC) for each species.

Species	Parameter	Relative Contribution (%)
Umbellularia californica	Elevation	47.78
(10m² grid resolution used)	Distance from ocean	20.54
	Soil type	14.31
	Degree slope	7.18
	Other	*10.18*
Rhododendron macrophyllum	Soil type	33.11
(10m² grid resolution used)	Distance from ocean	21.48
	Elevation	12.08
	Slope position	11.03
	Other	*22.30*
Lithocarpus densiflorus	Soil type	35.32
(30m² grid resolution used)	Distance from ocean	27.76
	Vegetation type	13.17
	Elevation	9.90
	Other	*13.84*

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Phytophthora ramorum - Pathogenic Fitness Among the Three Clonal Lineages[1]

Clare R. Elliott,[2] Virginia McDonald,[2] and Niklaus J. Grünwald[3]

Abstract

The Oomycete pathogen *Phytophthora ramorum* causes sudden oak death on oak and ramorum blight on a wide range of ornamental plants, causing severe economic losses to the nursery industry. The U.S. population of *P. ramorum* consists of three distinct clonal lineages referred to as NA1, NA2, and EU1. We tested the hypothesis of differences in fitness among these three lineages through the infection of detached leaves and whole plants in wounded and non-wounded inoculations of rhododendron, and also in *in vitro* experiments to assess growth and sporulation.

In independent experiments, the fitness of isolates within lineages was determined using the fitness components lesion area (LA), sporulation capacity (SC), incubation period (IP_w), and the area under the lesion expansion curve (AULEC) on wounded detached leaves of two cultivars of rhododendron. Three isolates from each clonal lineage were tested *in vitro*. In the non-wounded whole plant experiments, inoculation was achieved by dipping plants in a zoospore suspension of 5,000 zoospores/ml. Incidence was measured by the number of infections and the number of leaves infected, and severity was measured by the total lesion area and average lesion area per leaf. Ten *P. ramorum* isolates from each clonal lineage were studied *in vitro* to assess growth and sporulation at 10 °C and 20 °C dark incubation for 10 days using Petri plates of V8 100 agar and repeated twice.

Lesion area demonstrated significant differences among lineages in two out of three wounded detached leaf experiments; however, SC, IP_w, and AULEC showed no consistent significant differences among lineages. The non-wounded whole plant dip inoculations showed a trend towards a difference between the NA1 lineage and EU1 and NA2 ($0.1 > P > 0.05$), but variability among isolates within lineages means that these slight differences are not statistically significant. In one out of the two experiments on whole plants, significant differences between isolates within lineages were observed ($P < 0.026$). Analysis of variance on *in vitro* growth and sporulation of isolates of *P. ramorum* showed that at 10 °C there was a significant difference in the growth of isolates among lineages ($P < 0.001$) and at 20 °C there was a trend towards a difference in sporulation of isolates among lineages, but these differences weren't significant ($P = 0.075$).

Both *in vivo* studies and *in vitro* experiments all point towards slight fitness differences between clonal lineages of *P. ramorum*. Lineage NA1 generally grew more slowly, sporulated less, and infected at a lower frequency and severity than lineages NA2 and EU1. In many cases there was no significant difference between isolates of NA2 and EU1. In the whole plant, EU1 isolates demonstrated slightly higher incidence than NA2, but lower severity (not significantly different from NA1). More often than not these differences were not significant due to a high degree of variability among isolates within lineages. Variability was greater in lineages NA1 and EU1 compared with NA2 in *in vitro* growth and sporulation experiments.

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Susceptibility, Severity, and Sporulation Potential of *Phytophthora ramorum* on Several *Rhododendron* Species and Hybrids[1]

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Introduction

Several plant genera are important in the spread of *Phytophthora ramorum* in nurseries. These include *Rhododendron, Camellia, Viburnum, Pieris,* and *Kalmia*. In an effort to reduce the risk of *P. ramorum* being introduced onto their sites, some nurseries have reduced or eliminated these species from their inventory. Of these genera, *Rhododendron* is responsible for many of the *P. ramorum* positive finds in nurseries in the Pacific Northwest. With input from industry representatives, Washington State Department of Agriculture (WSDA), and the Rhododendron Species Foundation (RSF) we have identified approximately 100 *Rhododendron* hybrids and species to include in our testing. Three criteria were used to select species from the RSF collection for inclusion in this study: species that are native to the Yunnan province of China (it is speculated that *P. ramorum* may have originated from this area); species which are commonly cultivated; and species that are commonly used in hybridizing.

Methods

Leaves from 42 *Rhododendron* species were collected in November 2008 and February 2009 at the *Rhododendron* species garden, Federal Way, Washington (WA). A collection of 58 *Rhododendron* hybrids identified as being high priority for testing by the WA nursery industry was also sampled in November 2008. Treatments consisted of wounded and non-wounded inoculations with 10 µl of a zoospore suspension of *P. ramorum* (NA1 population) on the upper and lower leaf surfaces. A set of wounded treatments with a 10 µl drop of sterile distilled water was included for each species as a control. Leaves were placed on moist filter paper in Petri dishes and incubated at 19 C in the dark. Photos were taken at 5 and 10 days post-inoculation. Lesions were measured using APS ASSESS and lesion size expressed in mm^2.

Ten 6 mm diameter disks were cut from lesioned areas of leaves. If there was no lesion present, the area including the inoculum site was used. Disks were incubated in

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a Petri dish containing 1 ml sdH_2O and covered with nylon mesh to break surface tension. After 10 days, the disks were transferred to a 2 ml cryovial containing 1 ml sdH_2O and shaken on a tissue homogenizer for 40 s. The disks were removed from the spore suspension and spores were counted in five 30 µl aliquots placed on a microscope slide at 100 x. Both sporangia and chlamydospores were counted and spore counts were expressed as spores/ml and spores/lesion. The lesion area resulting from the wounded lower surface was used in these calculations.

Data for the fall 2008 and winter 2008 collections were analyzed using t-tests. *Rhododendron* species and hybrids were grouped into with/without indumentum, lepidote/elepidote, and species originating in Yunnan/other places. A correlation test was done comparing the fall and winter 2008 data for *Rhododendron* species.

Results

The wounded treatment produced larger lesions than the unwounded treatment. Infection frequency was also higher on the wounded treatment for both upper and lower leaf surfaces. Lesions were larger on the lower surface in both wounded and unwounded treatments, and infection frequency was higher on the lower surface. Species with indumentum on the lower leaf surface were less susceptible and produced smaller lesions than species without indumentum.

Rhododendron species and hybrids with scales on the lower leaf surface were more susceptible to infection and produced bigger lesions than those without, but the difference was not significant in the November 2008 sampling period for *Rhododendron* species. Species originating from Yunnan Province, China, were less susceptible to infection and had smaller lesions than those from other areas.

Sporulation was not influenced by presence of indumentum or scales, and there was no significant difference in sporulation between species originating from Yunnan and those from other places. There was a significant positive correlation between lesion size and sporangia/ml ($r = 0.509$, $P = 0.001$). There was no significant correlation between chlamydospores/ml and lesion size or sporangia/ml.

Preliminary data indicate that there is a great deal of variation among the *Rhododendron* species in our tests in the amount of sporangia and chlamydospores produced on the infected leaf discs. This may be related to leaf surface features such as indumentum and scales, and possibly chemical differences. Extremely high numbers of chlamydospores were produced on foliage of *R. campanulatum* (319/ml). *R. brachycarpum* produced the most sporangia (289/ml), more than 5 times the amount produced by *R. ponticum* (50/ml), the species responsible for spread of *P. ramorum* from European urban gardens to urban forests. Species that had low susceptibility to infection and low sporulation potential included *R. arboreum* and *R. keiskei*. *R. dauricum* was one of the most susceptible species and had high sporangia production (184/ml). Although additional testing is needed to confirm these results, it is clear that some rhododendrons pose a much higher risk of spreading *P. ramorum* than others.

Acknowledgments

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Phenotypic Variation in *Phytophthora ramorum*: Wild Type vs Non-Wild Type Isolates[1]

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Abstract

Phenotypic characteristics of four *Phytophthora ramorum* isolates with atypical culture morphology (non-wild type; *nwt*) were compared with four "wild type" (*wt*) isolates using material from stock cultures and after re-isolation from lesions on inoculated rhododendron leaves. Our preliminary results show that *nwt* isolates were more variable than *wt* isolates in all of the characters tested, and were generally lower in aggressiveness, chlamydospore production, and growth rate at all temperatures for both the original culture and when re-isolated from a host.

Introduction

In earlier studies, unusual culture morphology and behavior were noticed among some NA1 isolates of *Phytophthora ramorum*. This "non-wild type" behavior was not observed in our collection of isolates from the EU1 or NA2 lineages, even though the isolates had been in culture for a similar amount of time. It has been suggested that subculturing *in vitro* causes culture instability and loss of virulence, and passage through the host can revive the isolate back to its original state. To study this, we compared four less virulent isolates (non-wild type; *nwt*) with four isolates of normal virulence (wild type; *wt*) in our culture collection. One objective of this study was to determine whether *wt* behavior could be restored to *nwt* isolates of *P. ramorum* by successive re-isolation from host material.

Methods

Eight isolates of *P. ramorum* were selected and maintained on 15 percent V8 agar. Phenotypic characters examined on original cultures were pathogenic aggressiveness; growth rate at maximum, optimum, and minimum temperatures; and chlamydospore production in vitro. Detached leaves of *Rhododendron* "Cunningham's White" were inoculated with each of the isolates and lesion size measured using APS ASSESS,

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and then *P. ramorum* was isolated from lesions onto PARP and transferred to 15 percent V8 agar. These re-isolates were inoculated onto rhododendron leaves and re-isolated two more times, for a total of three successive re-isolations.

Growth rate at maximum, optimum, and minimum temperatures, and chlamydospore production were measured on cultures from the original and first re-isolation for each isolate.

Results

In both *wt* and *nwt* groups, there were significant differences in lesion size on detached rhododendron leaves between the original culture and the first re-isolation. Successive re-isolations were not different from the original culture and the first re-isolation. After re-isolation from the host, *nwt* isolates were still less aggressive than *wt* isolates. Along with lower aggressiveness on rhododendron leaves, *nwt* isolates produced fewer chlamydospores in V8 agar than did *wt* isolates. There was no difference in growth rate between the original culture and the first re-isolation for most isolates. However, *nwt* isolates were found to be more sensitive to temperatures below 2 °C and above 28 °C. The optimum growth temperature was 20 °C for both *wt* and *nwt* isolates.

Non-wild type isolates were more variable than *wt* in all characters tested. The greater variability suggests that these isolates are unstable or that slightly deleterious mutation(s) have accumulated in accordance with Muller's ratchet resulting in reduced fitness. *Wt* isolates performed better than *nwt* isolates in all of the phenotypic characters examined. Why *nwt* survives and proliferates is still a mystery. To understand the cause of these phenotypic differences, the role of cytoplasmic elements and differences in mitochondrial and nuclear DNA are being examined. Further studies will also include examining sporulation of *wt* and *nwt* isolates on plant hosts.

Acknowledgments

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Ecology of *Phytophthora ramorum* in Watercourses: Implications for the Spread and Management of Sudden Oak Death[1]

Elizabeth Fichtner,[2] Kamyar Aram,[2] and David Rizzo[2]

Abstract

Though stream-baiting has proven useful for early detection of new terrestrial infestations of *Phytophthora ramorum*, the biological and ecological rationale behind the success of baiting are unknown. Our current studies address the central theme of "why baiting works," focusing on specific questions including: i) What are the inoculum sources in streams? ii) Is there an inoculum threshold for baiting? iii) Can zoospore cysts undergo diplenetism to infect bait leaves? and iv) what are the trophic niches of *P. ramorum* in streams, and how does this influence bait detection?

To address the potential for *P. ramorum* to persist on aquatic or riparian plants, several plant species were collected from a *P. ramorum*-infested holding pond in Humboldt County, California. Aboveground plant parts and roots were baited with rhododendron leaf disks; however, thus far, *P. ramorum* was not recovered from any plant tissues. Additionally, aquatic plants have been inoculated to determine their susceptibility to *P. ramorum*, but the pathogen has not been re-isolated from any inoculated tissues. To assess the role of trophic niche on bait detection of *P. ramorum*, living and dead rhododendron leaves were incubated in two streams infested with the pathogen. *P. ramorum* colonized only living bait tissues, suggesting that the pathogen serves as a biotroph with respect to baiting in streams.

Laboratory experiments were designed to address the inoculum threshold necessary for bait detection and the potential for cysts to undergo diplanetism and subsequently infect baits. *P. ramorum* cysts, ranging in concentration (0, 10^1, 10^2, 10^3, 10^4), were placed in 1.5 ml microfuge tubes containing 1 ml of water, in the presence or absence of a surfactant. Cyst suspensions were centrifuged and then leaf disk baits were incubated on the water surfaces for 48 hours. Leaf disk baits were never infected in the presence of a surfactant. In the absence of surfactant, baits were successfully infected at cyst concentrations of 10^2 and 10^3 cysts/ml. The disparity between bait infectivity in the presence/absence of surfactant suggests that cysts may diplanetize, forming motile infective zoospores.

Preliminary results suggest that *P. ramorum* behaves as a biotroph when infecting bait materials in streams; however, the ability of *P. ramorum* to infect and persist on aquatic plants or colonize detritus in streams is unknown. Germination of cysts to form motile zoospores may aid in bait detection by enabling pathogen homing, thus enhancing bait efficacy at low inoculum concentrations.

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Emergence of *Phytophthora cinnamomi* in a Sudden Oak Death-Impacted Forest[1]

Elizabeth J. Fichtner,[2] David M. Rizzo,[2] Tedmund J. Swiecki,[3] and Elizabeth A. Bernhardt[3]

Abstract

The introduction of *Phytophthora ramorum* to China Camp State Park (CCSP) (Marin County, California) in the 1990s resulted in extensive mortality of *Quercus agrifolia* by 2000. However, mortality is now occurring in discrete disease centers among species of trees that were not affected by the sudden oak death (SOD) epidemic. This new mortality was first observed in long-term plots established by Phytosphere Research for study of SOD. Symptoms observed on Pacific madrone (*Arbutus menziesii*) included wilting followed by rapid mortality. In contrast, California bay laurel (*Umbellularia californica*) within affected areas typically showed thinning of the canopy and foliar chlorosis, followed by progressive top dieback. Most of the killed bay laurel observed to date have been saplings or small trees, whereas madrone mortality includes larger trees. Symptoms were not congruent with those associated with SOD, but were suggestive of a root disease.

Soil and root samples were collected beneath symptomatic trees. Roots were surface sterilized and embedded in PARP, whereas soil samples were baited with rhododendron leaf disks. *P. cinnamomi* was baited from multiple soil samples within the affected area and was isolated from roots of symptomatic bay laurel and madrone. *P. cambivora* was baited from soils in one portion of the affected area, but was not isolated from roots.

Containerized bay laurel and madrone were purchased for inoculation with *P. cinnamomi* and completion of Koch's Postulates. Pots of individual plants were placed in plastic bags to allow for periodic saturation of the container soil mix. Six plants of each species were inoculated by pouring a 15 ml suspension containing 10^5 zoospores/ml on the saturated soil surface; four plants of each species served as uninoculated controls. Pots were flooded for 24 hours after inoculation and then drained to container capacity. Pots were re-flooded for 24 hour intervals once each week over a 3-week period. The flood water was baited for *Phytophthora* during each flood event. After 3 weeks, roots were excavated from pots, surface sterilized, and baited with rhododendron leaf disks.

Bay laurel and madrone wilted within 2 weeks of inoculation with *P. cinnamomi*. Roots of inoculated plants were necrotic, and *P. cinnamomi* was re-isolated from symptomatic roots. In the initial run of this experiment, one of the non-inoculated control madrones died; *P. cactorum* was isolated from roots of this plant. In the second run of the experiment, *P. cinnamomi* was isolated from foliar lesions on a non-inoculated madrone. In subsequent trials, most non-inoculated plants remained asymptomatic; however, *P. cinnamomi*, *P. cambivora*, *P. cactorum*, *Pythium sterilum*, and *Pythium vexans* were isolated from surface-sterilized roots of non-inoculated container-grown madrone. *P. syringae* and *P. cinnamomi* were isolated from symptomatic foliage and stems of non-inoculated madrone. Additionally, *P.*

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nicotianae, *P. cryptogea*, *P. gonapodyides*, and *P. pseudosyringae* were baited from floodwater in madrone pots. *Pythium sterilum* was also isolated from non-inoculated bay laurel roots.

The results suggest that the recent mortality at CCSP is caused by *P. cinnamomi*. It is unknown when the pathogen was introduced, but a large patch of dead common manzanita (*Arctostaphylos manzanita*) and recently killed madrones were noted in the area in 2000. Over the past decade, *P. cinnamomi* has also been associated with disease in a natural oak woodland in southern California (Garbelotto and others 2006); mortality of Ione manzanita (*Arctostaphylos myrtifolia*), a rare plant limited to the unusually acidic Ione formation soils (Swiecki and others 2003); and several areas of madrone, California bay, and manzanita decline in the San Francisco Bay area.

Additional results of this work have demonstrated that *P. cambivora* and *P. cactorum* can form asymptomatic infections on madrone roots and *P. sterilum* forms asymptomatic infections on bay laurel roots. It is not known whether these asymptomatic infections may develop into root disease under different environmental conditions. Considering that all three organisms are associated with tree mortality or decline, the hidden transmission of these organisms as root inhabitants suggests a potential risk to susceptible hosts in both forest and nursery systems. Additionally, we demonstrated pathogenicity of *P. syringae* to herbaceous stems of madrone; however, infectivity by zoospores relied on presence of a pin-prick-sized wound. To date, *P. syringae* has not been isolated from madrone in California forest systems; however, it has been found to infect the related ornamental, *Arbutus unedo* (strawberry tree), in nurseries in Spain (Moralejo and others 2008).

The results of this study underscore the risk of pathogen transmission on infested containerized plants. Pathogens may be transported long distances on non-host plants, either in infested potting media, or as plant inhabitants associated with asymptomatic infections. For this study, infested plants were purchased from nurseries specializing in propagation of natives for habitat restoration plantings. The potential for introducing exotic pathogens through habitat restoration activities needs to be more widely recognized so that appropriate phytosanitary procedures can be applied to mitigate this risk to native plant communities.

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The Big Sur Ecological Monitoring Plot Network: Distribution and Impacts of Sudden Oak Death in the Santa Lucia Mountains[1]

Kerri Frangioso,[2] Margaret Metz,[2] Allison Wickland,[2] David Rizzo,[2] and Ross 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. It appears that no manner of preservation has been able to protect these wild areas from conservation threats such as exotic species (plants, animals, and pathogens). Big Sur has provided an exceptional environment to address questions about the ecological ramifications 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.

High-resolution, digital aerial photography integrated into a GIS was 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 ecological monitoring plots. Plots were stratified by forest type (mixed-evergreen and redwood-tanoak), level of tree mortality, watershed, fire history, and land ownership (public versus private). In 2006 and 2007, we established 280 long-term ecological monitoring plots throughout the region. Within each 500 m^2 circular plot, all stems greater than 1 cm diameter at breast height (dbh) were identified, measured, mapped, and scrutinized for *Phytophthora* symptoms and evidence of other pests. We also quantified the number and identity of regenerating seedlings and saplings, the percent coverage of each species, and plot-wide canopy height and openness, as well as topographical descriptors such as elevation, slope, and aspect.

In sum, we collected detailed information on over 13,400 trees throughout the Big Sur region. Of the 280 plots, 143 are on public land and 137 are on private land. There are 163 mixed-evergreen plots and 117 redwood-tanoak plots from Carmel Valley in the north to the Monterey County line in the south. Eighty of 163 mixed-evergreen plots and 73 of 117 redwood-tanoak plots tested positive for *P. ramorum* for a total of 153 plots out of the total 280 testing positive for the pathogen responsible for sudden oak death (SOD). Thirty-seven plots that did not have *P. ramorum* tested positive for other species of *Phytophthora*. Twenty plots had 100 percent infection of tanoak (*Lithocarpus densiflorus*) and a few plots had 100 percent infection of coast live oak (*Quercus agrifolia*). In *P. ramorum*-positive plots, many host species are living and showing potentially fatal canker or twig symptoms.

The mean standing dead basal area per plot in infested plots compared to uninfested plots is

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significantly higher for host species in both forest types. Total dead host basal area in the form of standing dead stems was 77 percent (p-value = 0.0001) higher in infested redwood-tanoak plots and 36 percent (p-value = 0.0005) higher in infested mixed-evergreen plots compared to background mortality levels in uninfested plots. Dead host basal area in the form of downed coarse woody debris (logs >20 cm diameter) was almost 3 times the amount for tanoak and 1.4 times for host oak species in infested plots compared to background mortality levels in uninfested plots. This metric not only suggests mortality levels above the standing mortality measured, but also has implications for nutrient cycling and fire affects.

Mortality from SOD has caused shifts in species abundance; infested stands are increasingly dominated by species such as California bay laurel (*Umbellularia californica*) that are not killed by the disease. There are significantly more bay stems in infested mixed-evergreen plots than in uninfested mixed-evergreen plots.

Mortality in positive plots was 13 percent for tanoak, 19 percent for coast live oak, and 12 percent for Shreve's oak (*Q. parvula* var. *shrevei*). However, much higher levels of mortality are occurring in certain stem size classes. For example, in tanoak, over three times the mortality is occurring in the larger size classes as compared to the smallest stem size class.

These plots provide invaluable information on environment, vegetation, forest structure, disease level, and site history in areas with and without the disease. 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 throughout the state of California. Furthermore, with the arrival of the 2008 fires in Big Sur and our extensive pre-fire dataset throughout our plot monitoring network, we are ideally situated to learn about the first wildfire in SOD-impacted wildlands.

Landscape Epidemiology of Species Diversity Effects on Disease Risk[1]

S.E. Haas,[2] M. Metz,[3] K. Frangioso,[3] D. Rizzo,[3] and R.K. Meentemeyer[2]

Abstract

Identifying environmental variables contributing to *Phytophthora ramorum* spread and persistence is critical to management and preservation of threatened forest ecosystems. Recent studies have shown that species diversity can affect disease risk via alterations in transmission potential between hosts. Most diversity-disease risk studies to date have focused on animals, with much less attention on generalist plant pathogens in natural ecosystems, and little has been done to incorporate spatial dimensions of landscape heterogeneity into such studies. Here, we examine diversity-disease risk in *P. ramorum*, focusing on the effects of species diversity within field plots and landscape patterns of disease existence and vegetative assemblages among plots throughout Big Sur, California. We include 'force of infection' in our regression models to account for inoculum exposure pressure from all known sources of disease throughout the study area. The analyses revealed a negative relationship between species richness (number of species; range: 1 to 11) within plots and disease risk. The force of infection covariate in our model accounts for most of the predictive power of disease risk, followed by California bay laurel (*Umbellularia californica*) prevalence per plot. Our findings agree with other research in multi-host disease systems in that high species diversity is more likely to decrease than increase disease risk. This result may be occurring in this disease system through two specific mechanisms—'encounter reduction' (reduced encounters between susceptible and infected host species as additional non-host species are included) and 'susceptible host regulation' (a reduction in the number of susceptible hosts as diversity increases), both of which are the focus of ongoing research. Ongoing research entails using structural equation modeling (SEM) to further elucidate the complex relationships among abiotic and biotic landscape heterogeneity variables and disease risk.

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Effects of Sudden Oak Death on Habitat Suitability for the California Spotted Owl (*Strix occidentalis occidentalis*) in the Big Sur Ecoregion[1]

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Abstract

Emerging infectious diseases are increasingly recognized as a major threat to wildlife. The invasive forest disease sudden oak death (SOD) has recently caused considerable mortality of oak (*Qurecus* spp) and tanoak (*Lithocarpus densiflorus*) trees in the Big Sur ecoregion of California, which may negatively impact a range oak forest obligate animal species. In this poster we examine the potential influence of SOD tree mortality on the occurrence of the declining California spotted owl (*Strix occidentalis*) in Big Sur forests.

Specifically, we test two alternative hypotheses using a combination of forest field plots, owl occupancy data, and multi-scale GIS analysis of habitat factors: 1) spotted owls are less likely to occur in forests with larger amounts of SOD tree mortality due to habitat loss and fewer food resources for its mammalian prey, or 2) abundant snags and coarse woody debris due to tree mortality enhances habitat for its prey and thus increases probability of spotted owl occurrence. Our multi-scale logistic regression modeling indicated that spotted owls are more likely to occur in forests with greater amounts of tree mortality, after accounting for forest structure, topography, and fire disturbance variables, and the explanatory power of the tree mortality-owl presence effect increases with increasing landscape extents up to 400 m radius measurement areas.

These possibly counterintuitive results suggest that SOD could actually benefit spotted owl populations, but we hypothesize only in the short term. Over time as this disease-induced wave of snags and coarse woody debris subsides, the spotted owl may be faced with less suitable forest habitat and fewer food resources for its prey.

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Influence of Nitrogen Fertility on the Susceptibility of Rhododendrons to *Phytophthora ramorum*[1]

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Introduction

Research information demonstrating the effects of various cultural practices on host susceptibility to *Phytophthora ramorum* is generally lacking and thus limits the development of an integrated approach to managing diseases caused by this pathogen in irrigated nursery systems. Because rhododendrons and azaleas have accounted for about 90 percent of the plants associated with *P. ramorum*-positive nursery finds in Washington State (as well as being the most important hosts of *P. ramorum* in Europe), their management in an irrigated nursery environment is critical to controlling the spread of this pathogen (Dart and Chastagner 2007). Nitrogen fertility levels have been reported to increase disease levels in some *Phytophthora* pathosystems (Halsall and others 1983), but no data is available for the *P. ramorum*-rhododendron pathosystem.

Methods

During 2008, we investigated the dynamics between nitrogen (N) application rates and the susceptibility of rhododendron cultivars 'English Roseum,' 'Cunningham's White,' and 'Compact P.J.M.' to *P. ramorum*. Plants were transplanted from 1 gallon to 3 gallon containers in a medium of 100 percent Douglas-fir (*Pseudotsuga menziesii*) bark with Micromax™ incorporated at the rate of 1.75 lbs/yd^3, placed on a gravel nursery bed, and watered as needed with overhead sprinkler irrigation. Before beginning the experiment, residual fertilizer in the media was depleted and three treatments of ammonium nitrate fertilizer at 100, 300, and 600 ppm N was applied in liquid form twice a week to each of eight plants per cultivar starting on June 2. With each N fertilization, phosphorus in the form of potassium phosphate (100 ppm) and potassium in the form of potassium sulfate (200 ppm), were applied. Commencing with fertilizer application, the plants were switched to a drip irrigation system. In early October, plant growth, visual quality, and leaf color were measured. Color of the adaxial (upper) leaf surface of two mature leaves from the most recent growth flush was determined quantitatively with a Minolta CR200b Chroma Meter (Minolta, Ramsay, New Jersey). The CIELAB coordinates, L*a*b*, were recorded and the chroma (C*) and hue angle (h°) were calculated (McGuire 1992). At the same time,

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two sets of fully mature, current season leaves from each plant were harvested for 1) determination of leaf tissue N content and 2) *P. ramorum* inoculations.

Six detached leaves from each plant were inoculated with zoospores from an NA1 lineage rhododendron isolate of *P. ramorum* (03-74-N10A-A, from *R.* x 'Unique') by pipetting a 10 µl drop of suspension with 568,000 zoospores/ml onto the lower leaf surface. The leaf tissue beneath drops on three leaves was wounded using an insect pin, while the tissue beneath each drop on the other leaves was left unwounded. Leaves were incubated in Petri plates with moist filter paper in the dark at 19 to 20 ºC for 10 days.

Results

As expected, foliage color, shoot growth, plant quality indices, and foliage N levels increased with N fertility. Observed leaf color correlated with measured leaf color and plants given higher rates of N were greener than those fertilized at lower rates. Fertility had no effect on root length or density. Foliar N concentration increased with N rate. Based on an overall analysis of lesion size after 10 days, there was a significant difference in the susceptibility of the three cultivars to *P. ramorum*. 'Compact P.J.M.' had the smallest lesions, while 'English Roseum' had the largest. Lesions developed on all the wounded and unwounded inoculation sites on the 'English Roseum' and lesion size increased with increasing nitrogen fertility. Nitrogen fertility had no effect on lesion size on the other two cultivars.

Acknowledgments

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Stream Baiting for Sudden Oak Death: Fluvial Transport and Ecohydrology of the Invasive Plant Pathogen *Phytophthora ramorum* in Western Washington State[1]

Regina Johnson[2]

Abstract

Phytophthora ramorum, a member of the water molds (Oomycota), spreads in water and survives unfavorable conditions in soil. The pathogen has been shown to travel 15 m in windblown rain, and as much as 7 km in flowing water, and to survive up to 8 months in soil. In forest settings in California, windblown rain poses a major dispersal agent for *P. ramorum*, picking up spores from tree tops. In Washington State, *P. ramorum* is a pathogen in nursery settings rather than forests. Nursery stock on which spores are produced tend to be very small plants, usually less than 1 m tall, and spores tend to be dropped to the ground rather than picked up by wind and rain. This dispersal pattern, along with the ability to survive in soil and to be transported in water, suggests that in nursery settings, soil, soil water, and streams may be critical dispersal agents for this invasive pathogen.

Phytophthora ramorum has been found on plants and in soil, potting mix, and surface waters in nurseries in Washington State. Two years of detection efforts by the Washington State Department of Agriculture (WSDA) are reviewed in this study. The WSDA baited six streams in 2006, and eight in 2007, for a total of 11 different streams. Of these streams, *P. ramorum* has been found in three. Positive stream baits in all three streams show a correlation with rising temperatures and decreasing precipitation in spring, particularly in April.

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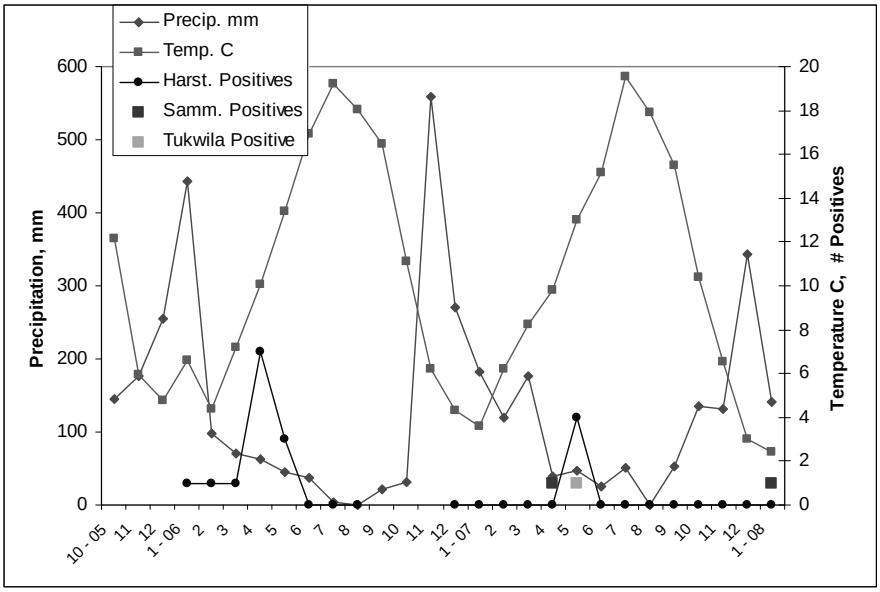

Figure 1—Precipitation, temperature, and positive stream baits for R Stream (Harstine), the Sammamish River, and Tukwila soil drainage ditch, 2006-07. Climate data for R Stream area from Western Regional Climate Center, http://www.wrcc.dri.edu. Climate data for Sammamish River/Tukwila ditch area are similar in pattern.

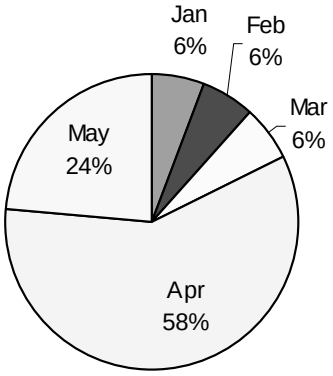

Figure 2— Positives in R Stream by month, 2006-2007.

Five *P. ramorum*-positive nurseries are compared by soil and hydrologic factors. Two are associated with positive streams, while the other three failed to produce *P. ramorum*-positive stream baits. Of these five nurseries, only one is associated with multiple positive stream baits over the 2-year period. This nursery has soil and hydrologic features distinct from all other nurseries studied. These features may be diagnostic for sites conducive to the escape of *P. ramorum* from the nursery environment to establishment in the wider landscape, in particular,

coarse, gravelly, shallow, sloped soil, and a low-order, high-gradient, gravel-bed stream.

Deep, fine, biologically active soils filter microbes, while shallow, coarse, biologically inactive soils allow microbes to pass through (Brady and Weil 2002). Shallow, coarse, Harstine soil may allow propagules of *P. ramorum* to pass through in soil water from the nursery into the stream. All other soils in this study are deep, fine-textured soils, which would be expected to filter and trap microbes in soil water.

Hardpans are known to concentrate and transport spores of *P. cinnamomi*, potentially as far as 120 m (Shea and others 1983, Kinal and others 1993). Both R Stream and the Tukwila soil drainage ditch positives are associated with a shallow hardpan. A natural hardpan underlies Harstine soil, while the Tukwila soil nursery has created an artificial hardpan by building raised display beds of composted shavings on top of muck soil. One soil-positive display bed is sloped towards the stream-positive drainage ditch. Shallow hardpan, coarse soil, and slope appear to be conducive to escape of *P. ramorum* from nurseries into streams. More data are necessary to examine the relative effects of these three factors.

Table 1—Nursery soil types with selected characteristics. Soil data from NRCS's Web Soil Survey.

Soil Series Name	General type	Slope, %	Hardpan	AWC*	Hydrologic group**	Coarse fractions
Harstine (R Stream), repeat stream positive	gravelly sandy loam, glacial till	6	yes	low	C	15-25%
Newberg/Nooksack	very fine sandy loam/silt loam, alluvial	0	no	very high/high	B/C	0
Terric Medisaprist (under composted shavings)	muck	0	artificial, at soil/shavings interface	moderate	ponded	0
Tukwila (under composted shavings), single stream positive, Samm. tributary	muck	0	artificial, at soil/shavings interface	high	D (ponded)	0
Woodinville	silty clay loam, alluvial	0	no	high	D (flooded)	0

* Available water content.
** Categorizes soil infiltration rate and runoff potential, with A = high infiltration/low runoff and D = low infiltration/high runoff.

While the nursery itself has apparently been cleared of *P. ramorum*, and no infested riparian plant material has been found, the stream continues to produce positive stream baits. Survival in damp soil has been shown to be sufficiently long for *P. ramorum* to survive Washington's dry season in stream sediments (Fitchner and others 2007). *P. ramorum* in the form of decomposing infected plant material could be stored in sediments in the channel, along the banks, and in floodplains, and could still be infective when remobilized by the stream. Stream sediments could be acting as reservoirs of infective material in the absence of infected plant hosts.

Acknowledgments

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Evaluation of Diurnal Rhythms in *Phytophthora*[1]

Takao Kasuga[2] and Mai Bui[2]

Abstract

A daily rhythmic activity cycle, or circadian rhythm, is an endogenously generated 24-hour periodicity, and can be entrained by external cues, such as daylight. These rhythms allow organisms to anticipate and physiologically prepare for precise and regular environmental changes. Many organisms, including plants, animals, fungi, and cyanobacteria, are known to display circadian cycles. In the fungal kingdom, rhythms in spore development and discharge are widespread, which indicate a selective advantage for regulation of these events at specific times of the day.

It is not known if oomycete pathogens possess endogenous circadian rhythm or whether they utilize metabolic cues from host plants to generate diurnal periodicity. It has, however, been shown that in the *Phytophthora capsici*-pepper hydroponic system, sporangium production and zoospore release were cyclic; sporangium production reaches a peak at 4 p.m., whereas zoospore release takes place at a maximum rate 2 hours after dark (Nielson and others 2006). Diurnal periodicity has also been reported for sporulation of downy mildew pathogens *Plasmopara viticola* (Rumbolz and others 2002, Yarwood 1937) and *Bremia Lactucae* (Nordskog and others 2007).

Furthermore, it is known that, for example, in fungi as much as 20 percent of the total gene transcripts display circadian periodicity. Because of this confounding effect, disregarding circadian periodicity while conducting global mRNA profiling or proteomics research, can potentially lead to misinterpretation of high throughput datasets. This has urged us to evaluate diurnal rhythmicity of *P. ramorum* and its potential link to pathogenicity using microarray mRNA profiling.

We grew *P. ramorum* under a diurnal photo cycle (12 hours light : 12 hours dark) for 6 days, then shifted to a 24 hour constant dark condition. Persistence of 24-hour periodicity in mRNA expression was then evaluated using fast Fourier transformation analysis. Out of 15,495 gene expression profiles obtained by *P. ramorum* NimbleGen microarray, only 94 genes (0.6 percent) showed persistent 24 hour diurnal rhythmicity. Because genes showing circadian rhythmicity in *P. ramorum* were much less than those found in ascomycete fungi, it is not clear if *Phytophthora* possesses an endogenous circadian system. Given the assumption that diurnal rhythmicity is essential to survival, *P. ramorum* might use cues of diurnal cycle from host plants. *Phytophthora* might be a circadian parasite.

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OakMapper 2.0: Distributed Participatory Sensing for Monitoring and Management of Sudden Oak Death[1]

Maggi Kelly,[2] John Connors,[2] and Shufei Lei[2]

Background

Public interest in sudden oak death (SOD), caused by *Phytophthora ramorum*, remains high as the disease continues to spread and impact more areas. Early in the infestation, information from active members of the public was key in locating new areas of infestation across the state. The California Oak Mortality Task Force (COMTF), arborists, and university researchers were repeatedly contacted with reports of new areas of suspected infestations. In response to this concern from the public, we created a website in 2001 where visitors could submit the locations of trees that were potentially infected. This site, OakMapper (www.oakmapper.org), has had thousands of visitors who have submitted hundreds of point locations of trees suspected of having the disease. In addition to this functionality, over time the first version of the OakMapper served as a clearinghouse for four SOD-related, spatial resources: 1) Google Maps, 2) Google Earth, 3) ESRI ArcIMS, and 4) static maps. All of these resources were dependent upon a project administrator to manually update their source data and reload the content to the website on a quarterly basis.

The OakMapper webGIS application is our comprehensive database and cartographic portal, containing all SOD data available for public viewing. In October 2008, we launched the second version of our webGIS, OakMapper 2.0, offering a more dynamic, customizable, and user-driven cartographic environment that is built on a combination of open-source and proprietary software (Kearns and others 2003, USDOC 2002). OakMapper 2.0 allows user-specific interactions – including scale-dependent zooming, customized map creation, hyperlinked photography, and querying functions – using the spatial database PostGIS. The webGIS site also allows users to report trees that might have the disease so that follow-up sampling can take place. 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 professional background, and 3) submit the location of the

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tree (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, Kelly and others 2004).

The New OakMapper

OakMapper 2.0 integrates the features of OakMapper 1.0 into one package and then further extends to other new features. As new open-source tools became available, we were interested in migrating to these more flexible solutions. The migration process begins with consolidating disparate data storage formats and sources, such as shapefile, MS Access, and Excel, into a single format and data source for which we selected SQL in the open-source PostgresSQL. The existing data is then migrated into a spatial database, enabled by PostGIS. PostGIS, which is an open-source spatial database, allows us to perform spatial data query and analysis. (Currently, OakMapper 2.0 is not utilizing the full features of PostGIS; this is set for future development.)

OakMapper 1.0 had four distinct and primary components: static maps, ESRI ArcIMS, Google Maps API, and Google Earth KML/KMZ. Instead of having them in an integrated system, the front page of OakMapper 1.0 functions like a portal web page for each of these four components. As a result, there is no navigation and interaction between these four components within the OakMapper 1.0 site. OakMapper 2.0 first integrates these four components by providing a navigation menu at the top. The navigation system allows users to travel back and forth among these components easily and provides a consistent feel and experience throughout the site. OakMapper 2.0 also allows different components to interact with one another. For example, the static maps can be selected for download using the Google Maps API download tool. Also, when you submit a point to the system via the Google Maps API, the Google Earth KML data file will be automatically updated.

OakMapper 2.0 (fig. 1) allows any users to come to the system and submit new findings of SOD to the database. Designing the system for the general public, the migration follows the user-centered design philosophy to achieve ease of use for end users. When reporting a suspected case of SOD, users simply 1) draw a point or a polygon on the Google Map and 2) enter relevant information, such as descriptions and even pictures about the new SOD find. This easy-to-use system is built to encourage community participation in recording more SOD occurrences, so that spread can be tracked more efficiently. And given that users' submissions are open to the general public, the public can be alerted about the new occurrences of SOD. The

Figure 1—OakMapper 2.0 website front page.

most recent SOD submissions will be displayed on the homepage, so that users can view the most recent activity on the site. The interaction between these features is enabled by their shared database.

OakMapper 2.0 allows users to register into the system so that they can keep track of their SOD submissions. Given that users might want to modify the descriptions or other information of their SOD submissions, registered users are provided with tools to edit their submissions. Registered users can also provide comments on SOD submissions. The commenting features of OakMapper 2.0 will facilitate more information generation and community building. Users can comment on the severity of SOD submissions. Like the submissions of SOD, users can keep track of and edit their submitted comments in the "My Account" section.

To improve the system's responsiveness to users' activities on the OakMapper 2.0 site, the system sends a confirmation email to the users when they register and when they submit an observation of SOD. The confirmation email will also contain the most recent SOD submissions and the most recent comments, which link back to OakMapper 2.0 for further exploration. RSS feed is a familiar tool in the Web 2.0 world. The GeoRSS standard provides a way to integrate RSS feeds with location information. OakMapper 2.0 generates GeoRSS feeds so that feed readers with spatial awareness can take advantage of the RSS feed of SOD submissions. The standard GeoRSS format allows the SOD data to be integrated with other web-map mashup applications.

OakMapper serves as an important resource for researchers to access the most up-to-date maps of confirmed cases of SOD. Our new WebGIS, built on ESRI ArcGIS Server, utilizes ArcSDE to reference the PostGIS spatial database to display the most up-to-date data available. This new structure ensures that users have access to all confirmed points and frees the site administrator from manually creating dozens of static maps.

Sign Up on OakMapper

The official map of sudden oak death in California shows only a few hundred individual trees with the disease. This is because of the time and expense required to officially confirm the presence of *P. ramorum*; the California Department of Food and Agriculture and the University of California perform this confirmation process on samples collected statewide. This map of individual trees does not show the complete extent of oak mortality statewide, and we are interested in getting public help in mapping other pockets of oak mortality that are not shown on the official map. Not all of these areas can or will be officially confirmed to have the disease, but we are interested in further defining where oak mortality exists, with your help. For example, there are many clusters of oak mortality in the East Bay Regional Parks that have not yet been mapped. OakMapper 2.0 can help. We would like you to use this tool to map areas where you see pockets of oak mortality that might be connected to SOD. We hope that this model of data acquisition, storage, analysis, and dissemination will be more widely used in forest health management in particular, and in natural resource management in general, while proponents of such a system will remain cognizant of the potential challenges.

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Quantifying Large-Scale Impacts of Sudden Oak Death on Carbon Loss in the Big Sur Basin Complex Fire: Upscaling From the Plot to Region[1]

Sanjay Lamsal,[2] Ross K. Meentemeyer,[2] Qingmin Meng,[2] Margaret Metz,[3] Richard Cobb,[3] Kerri Frangioso,[3] and David M. Rizzo[3]

Abstract

In California forests, sudden oak death- (SOD) related mortality and frequent wildfires have emerged as an important forest management concern. The forests of Big Sur are among the most impacted by SOD. Widespread tree mortality reduces biome production and carbon uptake, and increases future carbon emissions from decay and burning of coarse woody debris and dead trees. We hypothesize that SOD has a positive feedback on fire intensity and increases future carbon emissions, but the effects may be confounded by site physiography, fire characteristics (direction, intensity), and weather conditions. Our goal is to assess the interaction between SOD mortality, forest fire characteristics, and SOD contribution to aboveground biomass/carbon losses in the Big Sur region. We surveyed 280 plots, and estimated volume of coarse woody debris and biomass/carbon stored within live trees using their diameter at breast height. Post fire effects were surveyed in 61 burned plots to assess fire characteristics and their effects on coarse woody debris. Analyses are ongoing to estimate carbon losses from coarse woody debris across plots with different levels of mortality. Future surveys will focus on estimating SOD contributions to biomass/carbon losses from the plots and quantifying the large scale effects across the Big Sur region. Ignoring the effects of SOD on carbon dynamics and failure to account for the losses may overestimate the potential for forests to offset anthropogenic CO_2.

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The California Oak Mortality Task Force and *Phytophthora ramorum* Outreach[1]

Chris Lee,[2] Janice Alexander,[3] and Katie Palmieri[4]

Abstract

Since 2000, the California Oak Mortality Task Force (COMTF) has coordinated a comprehensive program of research, management, monitoring, education, and public policy related to *Phytophthora ramorum* and its impacts. In support of the COMTF mission, all five of these disciplines have education and outreach components. This education and outreach represents one of the first and most wide-reaching efforts to coordinate and provide information about a forest disease for disparate audiences, a task made even more complex by *P. ramorum's* occurrence in nurseries as well as forests.

The COMTF accomplishes these goals with the help of two outreach coordinators, a public information officer, an outreach associate, and a webmaster. These personnel are assisted on specific projects by additional university and agency staff around the state. Their efforts provide up-to-date science-based *P. ramorum*-related information to the 14 infested counties in California, as well as throughout non-infested areas in the state and the country, using educational materials via every major medium. This information is directed to the general public, land managers, other natural resource professionals, affected industries, regulators, policy makers, news media, educators, and scientific researchers. Additionally, the outreach and education staff assists in linking *P. ramorum* researchers throughout the world to each other by facilitating meetings, scientific symposia, and teleconferences.

One successful example of COMTF outreach and education work involves treatment and training workshops provided every year in a variety of communities throughout California. Some of these workshops, given in cooperation with the University of California, Berkeley Garbelotto laboratory, give participants an opportunity to closely observe techniques for treating trees and landscapes to prevent or lessen the impacts of *P. ramorum* on their properties or in their communities. Another series of repeating workshops brings together *P. ramorum* researchers, educators, and ecologists from throughout California to give varied audiences the latest information on *P. ramorum* biology, distribution, monitoring, management, and regulations.

Other notable examples of outreach and education efforts include the creation and maintenance of a website that serves as the main nexus for easy-to-find information on *P. ramorum*; the facilitation of numerous local meetings to discuss *P. ramorum* impacts in specific communities; a monthly newsletter that summarizes the latest *P. ramorum*-related findings and information; the compilation of *P. ramorum* information and the coordination of monitoring efforts with local tribes; communication with media; and the fielding of daily phone calls concerning *P. ramorum* and related forest health issues from the public. Periodic self-assessment through public surveys helps to refine outreach efforts and guide the development of future efforts so that the dissemination of new information improves in tandem with our own increased knowledge of the pathogen.

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Oregon's Grower Assisted Inspection Program: an Audit-Based System to Manage *Phytophthora* Diseases[1]

Melissa Lujan,[2] Gary McAninch,[2] and Nancy Osterbauer[2]

Introduction

Invasive plant pathogens are a tremendous threat to forest health. *Phytophthora ramorum*, an exotic fungus-like organism, attacks 118 plant species and kills mature oak, tanoak, and beech trees. It is established in 14 counties in California and is found in a limited area near Brookings, Oregon. The pathogen is also a problem in nurseries, where it can infect plants and infest soil and irrigation water. In Europe, it has been shown that *P. ramorum* can spread from infected nursery stock into natural landscapes. A federal quarantine regulates the movement of *P. ramorum*-susceptible plants within the U.S. However, the pathogen continues to be detected in plants moving through the nursery trade. The Oregon Department of Agriculture (ODA) worked with Oregon's nursery industry and others to develop a systematic approach to managing *Phytophthora* problems, including *P. ramorum*, in nurseries. The voluntary Grower Assisted Inspection Program (GAIP) is designed to compliment the federal quarantine by enlisting the cooperation of nurseries in preventing the spread of *P. ramorum* through the movement of infected plants. The nurseries do so by adopting best practices for *Phytophthora* disease management.

Program Structure

Education Requirements

With the help of Oregon State University (OSU) and the U.S. Department of Agriculture, Agricultural Research Service (USDA ARS), a bilingual online training course (http://ecampus.oregonstate.edu/phytophthora) that describes *Phytophthora* biology, best management practices for *Phytophthoras* in nurseries, and *P. ramorum* specifically was developed. All GAIP participants had to take and pass this course. A workshop was held to train nursery workers to identify *Phytophthora*-infected plants in the field and test them using the *Phytophthora* Alert®-LF field kit (Neogen Europe, Ltd.).

Hazard Analysis

Each nursery was required to review their production and procurement processes to

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determine where *Phytophthora* could be introduced into their operation. OSU and ARS research identified three critical control points (CCP) where a *Phytophthora* could be introduced into a nursery - water, soil and/or potting media, and used containers (Parke and others 2008). The ODA identified a fourth CCP for *P. ramorum* - incoming plants. Nurseries were encouraged to include other CCP unique to their operation.

Mitigation Manuals

Once all CCP were identified, each nursery had to identify best management practices that would mitigate the risk of *Phytophthora* being introduced at that CCP. Each practice had to be auditable through documentation, interviews, or observations. The nursery documented these practices in a mitigation manual that was then reviewed by the ODA.

Nursery Audits

Once their mitigation manual was accepted, each nursery was audited at least three times per year. The first audit ensured the nursery had the necessary infrastructure to carry out the best practices outlined in their manual. The second and third audits were done to verify the nursery was actually following their manual. Host and associated plants (HAP) were inspected and sampled during the second and third audits.

Corrective Actions

If a nursery is out of compliance with their mitigation manual, they must implement a corrective action or be suspended from the GAIP. Major non-compliance issues result in immediate suspension, while minor non-compliance issues must be corrected within a set time period or the nursery will be suspended. Once additional audits verify corrective action has been taken, the suspension is lifted.

Quantifying the Impact of GAIP

Eighteen nurseries volunteered to participate in the GAIP. Although all nurseries are welcome to participate, education and outreach efforts focused primarily on growers of *Rhododendron* and *Camellia*, the hosts reportedly infected with *P. ramorum* the most often (Tubajika and others 2006).

To quantify the impact of the GAIP, we used the results of the federal certification survey (7 CFR 301.92), which requires all growers shipping HAP interstate to be inspected and have at least 40 samples tested annually for *P. ramorum*. The ODA surveys *Rhododendron* and *Camellia* growers twice each year as a matter of internal policy.

During each inspection, samples were collected if suspicious symptoms were observed. One sample consisted of five individual, symptomatic leaves collected from a single plant. Sampled plants were flagged for later identification. Samples were initially screened for *Phytophthora* using a commercially available ELISA kit (Agdia, Inc., Elkhart, IN). Samples positive with ELISA were then tested using the

USDA-approved molecular diagnostic protocols (http://www.Aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml).

Results and Discussion

In 2007, the ODA inspected all growers/shippers of *P. ramorum*-susceptible plants to establish a baseline for aerial *Phytophthora* species in Oregon nurseries. *Phytophthora* were detected in 42.0 percent of the 754 sites surveyed, with *P. ramorum* detected at three sites. Of 29,665 plant samples tested, 1,480 were infected with a *Phytophthora* and four of those with *P. ramorum*. This showed that aerial *Phytophthora* are a common disease problem in Oregon nurseries, while *P. ramorum* is present at a very low level.

In 2007, *Phytophthora* was detected at 17 of the 18 GAIP nurseries (94.4 percent). The percentage of samples found infected with *Phytophthora* was used as a measure of the level of *Phytophthora* disease present in each nursery. In 2007, the average level of disease detected within the nurseries was 14.6 percent. In 2008, *Phytophthora* was detected at 16 of the 18 GAIP nurseries (88.9 percent), while the average level of disease detected was 14.9 percent. From 2007 to 2008, the amount of disease detected decreased significantly at three nurseries, remained the same at 11 nurseries, and increased significantly at four nurseries (fig. 1, $p = 0.10$). As of June 2, 2009, *Phytophthora* was detected in five of seven nurseries surveyed (71.4 percent), while the average level of disease detected was 8.0 percent. From 2008 to 2009, the amount of disease detected decreased significantly at four of the seven nurseries surveyed. In the remaining nurseries, the disease level remained the same. Many of the volunteers were adopting new best practices at their sites in 2008. We believe the 2009 survey results will provide a better picture of the impact of the GAIP on aerial *Phytophthora* species within these nurseries.

We also examined which hosts were found infected within the GAIP nurseries. In 2007, *Phytophthora* was detected on 17 host genera. *Phytophthora* was found on *Rhododendron* and *Pieris* the most often, representing 74 percent and 7 percent of all infected samples, respectively. In 2008, *Phytophthora* was detected on fewer host genera (13). Again, *Phytophthora* was detected on *Rhododendron* (82 percent of all infected samples) and *Pieris* (7 percent of all infected samples) the most often. Preliminary results from 2009 support these earlier findings. Tubajika and others (2006) reported *Rhododendron* and *Camellia* were at highest risk for spreading *P. ramorum*. Our results suggest that in Oregon, disease management and outreach efforts would be better focused on *Rhododendron* and *Pieris* growers.

Further research by OSU and ARS scientists has shown that there is a substantial endemic *Phytophthora* population present in many nurseries' soil substrate (Jennifer Parke, Oregon State University, Corvallis, OR, personal communication). Many of the practices adopted by the GAIP nurseries, such as preventing contamination of potting media with native soil, address this issue indirectly. Further work needs to be done to identify best practices that directly address the issue of endemic *Phytophthora* populations in soil. Therefore, it may take several growing seasons for new, best practices to have an impact at nurseries with significant, endemic populations of *Phytophthora* in their soil substrate.

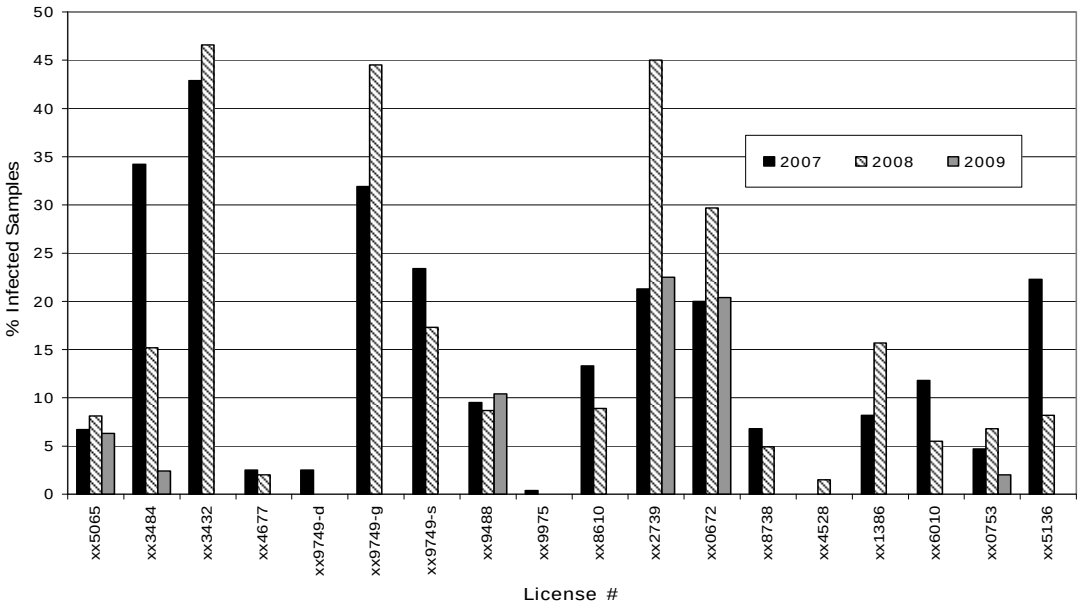

Figure 1—The percentage of aerial *Phytophthora* disease present at GAIP nurseries in 2007, 2008, and 2009.

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Effect of Plant Sterols and Tannins on *Phytophthora ramorum* Growth and Sporulation[1]

Daniel Manter,[2] Eli Kolodny,[2] Rick Kelsey,[3] and Pilar González-Hernández[4]

Abstract

The acquisition of plant sterols, mediated via elicitins, is required for growth and sporulation of *Phytophthora* spp. In this study, we examined the effect of plant sterols and tannins on growth and sporulation of *Phytophthora ramorum*. When ground leaf tissue was added to growth media, *P. ramorum* growth and sporulation was greatest on California bay laurel (*Umbellularia californica*) as compared to either California black oak (*Quercus kelloggii*) or Oregon white oak (*Q. garryana*), which is in agreement with field observations. However, when purified foliar sterol extracts were added to the media, no difference in growth and sporulation of *P. ramorum* was observed, suggesting the presence of an inhibitor in the foliage of the two oak species. Tannins are polyphenolic compounds that have the ability to precipitate proteins and are found within a wide array of plants, particularly oak species. Foliar tannins from all three plant species were able to bind and precipitate elicitins, and a linear relationship was observed between the amount of elictin removed (precipitated) by the tannins and *P. ramorum* growth and sporulation. Based on these studies, we suggest that the higher tannin content of oaks inhibits *P. ramorum* growth and sporulation by inactivating elicitins and sterol acquisition by *P. ramorum*.

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The Santa Lucia Preserve: A Case Study of Community-Based Sudden Oak Death Sampling Blitzes[1]

Cheryl M. McCormick[2] and Matteo Garbelotto[3]

Abstract

The Santa Lucia Preserve is a 20,000 acre, private, upscale conservation and limited development project in Carmel, California. In early 2007, Preserve residents, in collaboration with Dr. Matteo Garbelotto, developed the concept of a "SOD (sudden oak death) Blitz," whereby communities conduct a day-long sampling of California bay laurel (*Umbellularia californica*) leaves, which are subsequently analyzed for the presence of *Phytophthora ramorum* by Dr. Garbelotto's plant pathology laboratory at the University of California (UC), Berkeley. California's first SOD Blitz (coined "Bay Watch") was held on the Preserve in May 2008 and was attended by 67 Preserve landowners, certified arborists, and Preserve staff. Outcomes of the event included a map of the distribution of SOD on the Preserve, a comprehensive SOD management plan for the property, a series of SOD workshops, a "SOD Blitz Manual," and a prioritized strategy for managing SOD on the Preserve.

By partnering with Preserve landowners, the Santa Lucia Conservancy has developed a highly efficient protocol for successfully organizing and implementing a large-scale SOD Blitz. A team of landowner "Blitz Captains" is responsible for educating their neighbors about SOD and the Conservancy's management efforts, encouraging participation in Blitz events, and participation in numerous pre-event activities, such as sample protocol training, pre-flagging California bay laurel trees for sampling, and assembling sample kits. This 'peer-to-peer' education and outreach is highly effective in motivating landowners to participate in SOD Blitz events, and also has the added benefit of revealing critical information gaps that exist within the community, as Preserve community members are more likely to share their concerns and needs with each other rather than with a member of the Conservancy staff. This information, in turn, assists the Conservancy in developing workshops, educational resources, and priorities for management. A total of 253 samples were collected during the inaugural 2008 SOD Blitz, of which 42 (18.2 percent) tested positive for *P. ramorum* using conventional microbial and molecular techniques. Because each sample was referenced by a spatial coordinate transferred from a marked paper map to a GIS database using ArcView 9.3 (ESRI®), positive results can be mapped as discrete points buffered by a zone representing the average dispersal distance of *P. ramorum*. The area within these buffered distances represents the area within which active management should be considered.

As a direct result of the SOD Blitz efforts, an early detection and rapid response (ED&RR) team effectively eradicated two nascent foci of *P. ramorum* infection in previously uninfested habitats on the eastern portion of the Preserve. Landowners whose property was determined to contain a positive result were advised to consider a two-pronged approach to managing SOD, involving preventative treatment of bark host species (namely, coast live oaks, *Quercus*

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agrifolia) with Agri-Fos/Pentrabark® and selective removal and/or pruning of California bay laurel trees.

The Preserve's second annual event ("SOD-ZILLA 2009") was held on March 21, 2009, and was attended by 34 members of the Preserve community. Results are expected from the Garbelotto plant pathology laboratory at UC Berkeley in September 2009. A map depicting changes in the distribution of SOD on the Preserve from 2008 to 2009 will be produced and incorporated into the Conservancy's SOD management plan.

The Santa Lucia Conservancy's successful SOD Blitz protocols and organizational structure served as a template for a region-wide SOD Blitz, which was held on May 3, 2009 and encompassed over 37,000 acres from Carmel Valley south to middle Big Sur in Monterey County. Partners in the regional sampling effort included the Monterey Regional Park District, Big Sur Land Trust, White Rock Club, and several private landowners. The regional SOD Blitz was advertised in a number of local media outlets and attracted 23 general public participants from the Monterey Peninsula. A 1-hour workshop was held 2 weeks in advance of the event, during which participants were taught to reliably identify California bay laurel trees and SOD symptoms, and implement the simple sampling protocol. Sampling kits containing a step-by-step sampling protocol, data sheet, printed aerial photo with infrastructure layers, and 20 Ziploc® sandwich bags for sampled California bay laurel leaves were distributed to workshop participants, in addition to educational information and a complimentary phytosanitation hiking footwear bag. As with results for the second annual Preserve-based SOD event, results for the regional event are expected in September 2009 and will be made available via the Conservancy's website at: http://www.slconservancy.org.

Through the development and implementation of the SOD Blitz events, the Santa Lucia Conservancy, in collaboration with Dr. Matteo Garbelotto and local conservation partners, has made significant strides in advancing SOD awareness as a community-based dynamic as well as a private property concern.

The SOD Blitz events have spurred additional interest in other aspects of SOD management on the Preserve, such as phytosanitation, prevention and treatment, and research. As a result, the Conservancy has installed seasonal sanitation stations at the heads of frequently used trails, host treatment workshops, and sponsors field research advancing the frontiers of our ecological knowledge of *P. ramorum* and its life cycle in coastal forests. Additionally, the Conservancy has developed the State's first SOD management plan for private property, entitled, "Sudden Oak Death on the Santa Lucia Preserve: A Community Approach," which is available upon request.

As with the management of other non-native pests such as plants, long-term datasets are essential in tracking changes in the distribution and severity of bioinvasions so that predictions may be made about their containment and treatment. The SOD Blitz events provide a low-cost, reliable, educational, and predictable source of occurrence data with which to monitor the spread and life cycle of *P. ramorum* in California's coastal forests.

Ambrosia Beetles and Their Associated Fungi Appear to Accelerate Mortality in *Phytophthora ramorum*-Infected Coast Live Oaks[1]

Brice A. McPherson,[2] David L. Wood,[2] Nadir Erbilgin,[3] and Pierluigi Bonello[4]

Abstract

Infection of coast live oak (*Quercus agrifolia*) by *Phytophthora ramorum*, cause of sudden oak death (SOD), is consistently followed by bark and ambrosia beetle attacks on the bark overlying cankers. These beetles do not typically attack asymptomatic trees exhibiting healthy green crowns. Beetle attacks reduced median survival of infected coast live oaks by 65 to 80 percent compared with beetle-free trees (McPherson and others, these proceedings). This study was designed to explore the role of beetles and fungi in SOD and to determine the sequential appearance of different fungal species.

We inoculated coast live oaks with *P. ramorum* in March 2005 at two sites in Marin County, California, and then cut groups of infected trees at 6-month intervals thereafter. Asymptomatic trees were also felled and treated as controls. Bolts were cut from logs and dissected in the laboratory. From sections taken through these bolts at 15-cm intervals, surface-sterilized wood samples were cultured on three growth media. We separated and purified distinct morphotypes and amplified the internal transcribed spacer region (ITS) of the rDNA operon. Amplicons were sequenced and blasted in GenBank. Only isolates showing >95 percent ITS identity are reported here.

The diversity of fungal taxa isolated increased with time after inoculation and was considerably greater in trees that had been attacked by ambrosia beetles. Several of these fungi are known to be associated with *Quercus* spp., including *Arthrographis cuboidea, Botryosphaeria corticola*, *Pezicula cinnamomea*, and *Geosmithia langdonii*. Other fungal taxa, including *Botryosphaeria sarmentorum, Kabatiella microsticta*, *Stereum hirsutum*, *Trametes versicolor*, and *Truncatella angustata,* have been reported to be pathogenic to various hardwood species.

Beetles were reared from colonized trees, as well as dissected from their galleries, and were cultured on the same media. From the bark beetle *Pseudopityophthorus pubipennis*, we isolated *Botryosphaeria corticola, Geosmithia pallida, Hypocrea schweinitzii, Mucor racemosus,* and two molds. Fungi were isolated from four ambrosia beetle species: *Xyleborus californicus* (*Hypocrea lixii*, and two molds), *Monarthrum scutellare* (*Hypocrea viridescens*), *Monarthrum dentigerum* (*Lecanicillium* cf. *Psalliotae*), and *Xyleborinus saxeseni* (*Pestalotiopsis* sp.).

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Geosmithia spp. are found in association with bark beetles, including some that colonize *Quercus* spp. in Europe (Kolarik and others 2005). An undescribed *Geosmithia* species has been recently implicated in "thousand cankers disease," an apparently introduced beetle-vectored pathogen of walnuts in the western United States (Freeland and others 2009). The significance of the association of a closely related *Geosmithia* species with *P. ramorum*-infected coast live oaks is unknown.

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Mapping the Distribution of Sudden Oak Death at the Urban-Wildland Interface: Tilden Park in Berkeley, California[1]

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

Abstract

In coastal California, sudden oak death is primarily a disease of forests. Prior to 2001, the *Phytophthora ramorum* epidemic had not been found in the East Bay. This area east of San Francisco Bay, though heavily urbanized, has extensive forested parklands and protected watersheds that are similar in vegetation and climate to the heavily infested parts of Marin County. Following several years of isolated reports of infected coast live oaks (*Quercus agrifolia*) and California bay laurels (*Umbellularia californica*) in the East Bay, an extensive outbreak was discovered in Tilden Park, adjacent to Berkeley, in October 2006.

Tilden Park lies at an urban-wildland interface, in an area that has been the site of several major wildfires in the past century. As development continues to encroach on forested land, pressures increase on these resources. In collaboration with the East Bay Regional Park District, we are using the point-centered-quarter population density estimation method to determine infection levels in a geographic information systems (GIS) context and to map the distribution and variation in infection levels in coast live oaks within this park. This work is part of a larger project to assess the extent of the epidemic in the regional parks and to identify local factors influencing this distribution. A further goal of the study is to project the forest composition and structure resulting from the predicted loss of coast live oaks.

Preliminary results indicate that coast live oak infections caused by *P. ramorum* are distributed very unevenly across the park landscape. Individual coast live oak stands in the area near the outbreak that was identified in 2006 have infection levels between 22 percent and 61 percent. These levels are comparable to those observed in Marin County research sites in 2000 (McPherson and others 2005). Other coast live oak stands in the park are currently showing negligible infection levels.

Nearly two centuries of shifting land use patterns have created a patchwork of native vegetation and introduced species, interspersed with buildings, recreational areas, and roads. This heterogeneous landscape presents opportunities to understand the limits of the epidemic in the context of multiple factors that influence its local abundance and expansion. The resulting distributional maps will be designed to provide information to the land managers.

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Mapping the Impacts of Sudden Oak Death Tree Mortality on Severity of the Big Sur Basin Complex Fire[1]

Qingmin Meng,[2] Sanjay Lamsal,[2] Emily Holland,[2] Douglas Shoemaker,[2] Margaret Metz,[3] Kerri Frangioso,[3] David M. Rizzo,[3] and Ross K. Meentemeyer[2]

Introduction

Recent disturbance events, both biological and physical, continue to shape the heterogeneous landscapes of California's Big Sur ecoregion. Over the past decade, Big Sur has experienced substantial mortality of oak (*Quercus* spp.) and tanoak (*Lithocarpus densiflorus*) trees due to the emerging forest disease sudden oak death (SOD). In 2008, a series of large wildfires burned a substantial portion of the region, including a plot network established to study the spread and impacts of SOD. Spatially-explicit maps of pre-fire tree mortality (Meentemeyer and others 2008) and the existence of pre- and post-fire plot data provided an ideal opportunity to examine feedbacks between landscape heterogeneity, mortality-related fuel loads, and fire severity.

To examine landscape-level impacts of SOD on fire severity, we acquired hyperspectral MASTER (MODIS/ASTER) and AVIRIS remote sensing data of the burned area immediately following suppression of the wildfires. Of the 122 monitoring plots located within the burn perimeter, we quantified fire severity in 61 of the plots using BAER assessment methods prior to fall rains. Around each plot, we also measured pre-fire landscape heterogeneity of vegetation type, tree mortality, topography, and weather factors at the time of fire. These data were integrated with our hyperspectral imagery to upscale plot-level burn indices to regional maps of fire severity and to quantify the large-scale contribution of sudden oak death tree mortality to fire severity.

Methodology

Multivariate analysis methods such as multivariate correlation, principal component regression (PCR), and partial least squares regression (PLSR) have been applied in a wide range of fields including environmental science, natural resources, ecology, and geography. The main reason is that they have been designed to confront the situation that there are many, possibly correlated, predictor variables, and relatively few samples. This typically is a common situation in ecology, especially landscape

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epidemiology and fire ecology, in which extensive and on-time field surveys are time consuming and people often do not have enough time and financial support to complete large samples.

Principle component regression (PCR) and partial least squares regression (PLSR) provide the potential approaches which can explore the maximum meaningful information in hypespectral images and input the extracted information into fire burn severity prediction and mapping. Another advantage of PCR and PLSR is that a number of response variables (for example, burn severity of different forest stands' layers) can be modeled or predicted at the same time.

Results

Using the hyperspectral images MASTER, we regionally predicted and mapped fire severity of dominant tree layer, intermediate-sized tree layer, shrub layer, herb layer, and composite burn index across the mixed oak and redwood-tanoak forests in Big Sur, California. Our predictions for dominant tree layers are moderate strength, but predictions for intermediate-sized tree layer and the overall burn index (CBI) are relatively weak.

There are significant differences of fire severity across the Big Sur Complex Fire. For example, multivariate analyses indicated that differences of fire severity between mixed oak and redwood-tanoak forests are significant at the 0.005 level. After accounting for topographic and vegetation community type, SOD mortality mapped by Meentemeyer and others (2008) was positively correlated with fire severity.

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Phytophthora ramorum in USA Streams From the National Early Detection Survey of Forests[1]

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

Abstract

The National *Phytophthora ramorum* Early Detection Survey of Forests used terrestrial vegetation survey protocols from 2003 to 2006. The pathogen was detected in only two out of 12,699 symptomatic plant samples collected and diagnosed in 39 states. Stream surveys utilizing rhododendron leaf baits had been used successfully for *P. ramorum* early detection and monitoring in California and Oregon forests since at least 2004 and were examined as an alternative survey method in an effort to improve detection efficiency. The assumption that stream survey by baiting is more efficient than terrestrial survey was supported by the detection of the pathogen from 6 to 25 km downstream from the nearest known forest infestations even before symptoms were detectable in low-altitude aerial surveys. Successful pilot testing of stream baiting survey protocols in 11 states during 2006 resulted in full implementation in 2007, and stream baiting surveys have continued to the present.

Stream baiting surveys were conducted by cooperators in state agencies and universities. The number of streams surveyed in each state depended on risk as determined by host type, climate, and potential for *P. ramorum* introduction. In the pilot survey year, four non-wounded rhododendron leaves in a mesh bag were deployed in each stream at monthly intervals for five months during the growing season (May to September); leaves were exposed for 1 or 2 weeks each month, depending on symptom development. After retrieval, leaves were washed under running tap water and blotted dry, and then small pieces of water soaked or discolored leaf tissue were removed and assayed for *Phytophthora* species and *P. ramorum* by two methods—PCR and isolation on selective medium. Methods were modified slightly in later years. In 2007, two bags of bait leaves were deployed in each stream during each baiting period to compensate for occasional losses and to provide sufficient material for diagnostics. In 2008, a sixth baiting period was added with direction to avoid deployment in mid-summer months in states where high temperatures were presumed to be less favorable for growth and sporulation of *P. ramorum*. Results are presented for the period from 2006 through the first half of 2009.

Stream baiting surveys were completed in 320 unique streams in 28 states from 2006 through 2008 (table 1) with *P. ramorum* detected in waterways outside of known infested West Coast counties each year. The first detection was reported during the first baiting period of the 2006 pilot survey year in a seasonal stream draining a positive nursery in Pierce County, Washington. In 2007, this find was followed by a new detection in a river in King County, Washington with multiple positive nurseries in the watershed and in a ditch and creek outside

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a positive nursery in Rankin County, Mississippi. Seven more new detections were reported during 2008. Previously non-infested watersheds in the *P. ramorum*-endemic California counties of Mendocino (three streams) and Humboldt (one stream) and Curry County, Oregon (one stream), were found positive as well as waterways outside positive nurseries in Shelby County, Alabama and Gadsden County, Florida (one stream each). In the first half of the 2009 survey season, new detections have been reported in waterways outside positive nurseries in Montgomery County, Alabama and in Georgia (one each).

Table 1—Stream survey totals by region and year of survey

Region of USA	Number of states	Year of survey			Number of streams surveyed	
		2006	2007	2008	Total	Unique[a]
West Coast	3	37	32	39	108	75
South	10	33	64	71	168	137
North Central	6	0	20	15	35	28
Northeast	9	24	37	29	90	80
National Total	28	94	153	154	401	320

[a] Some streams were surveyed in multiple years.

In total, 12 first detections of *P. ramorum* have been made in seven states by the National Early Detection Survey of Forests in 3.5 years using rhododendron leaf baiting of waterways. Of these, five were made in streams draining watersheds near or adjacent to known positive watersheds within the known range of *P. ramorum* and sudden oak death in West Coast forests. The remaining seven streams are outside the known range but drain watersheds with one or more confirmed positive woody ornamental crop nurseries (table 2). To date, implementation of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service Confirmed Nursery Protocols in the infested nurseries have not prevented *P. ramorum* from escaping nursery environments, as the pathogen has been found repeatedly in waterways outside of these nurseries each year after initial detection and regulatory action.

Table 2—Location and year of *P. ramorum* detections outside the known West Coast range of sudden oak death, January 2006 through June 2009

Location	Year of detection			
	2006	2007	2008	Jan-Jun 2009
Pierce Co., WA	X	X[a]	X	X
King Co., WA		X	X	X
Rankin Co., MS		X	X	X
Gadsden Co., FL			X	X
Shelby Co., AL			X	X
GA				X
Montgomery Co., AL				X

[a] Detection made by Washington State Department of Agriculture in a bait station not part of the National Early Detection Survey of forests.

While asymptomatic plants, contaminated soil, and/or contaminated water inside confirmed positive nurseries are strongly implicated as the sources of inoculum detected in these streams, the possibility exists that exterior sources also are present. Repeated intensive vegetation surveys in the environs of positive streams in Washington, Mississippi, Alabama, Florida, and Georgia resulted in positive diagnoses of *P. ramorum* only in Mississippi during winter 2007-2008, when four PCR-positive results were obtained from samples of three different host and associated plant genera collected on two different survey dates. Stream surveys using rhododendron leaf baits will continue, and intensive vegetation surveys in the environs of positive streams will be repeated in order to ensure that *P. ramorum* has not become established in natural ecosystems outside the currently known range of this pathogen.

The *Phytophthora* Online Course: Training for Nursery Growers[1]

Jennifer L. Parke,[2] Jay Pscheidt,[3] Richard Regan,[4] Jan Hedberg,[5] and Niklaus Grünwald[6]

Abstract

Oregon State University Extended Campus (Ecampus) launched an online training course for the management of *Phytophthora* in nurseries. The course was developed with funds from the U.S. Department of Agriculture's Natural Resource Conservation Service through a grant to the Oregon Department of Agriculture. To access the *Phytophthora* Online Course: Training for Nursery Growers, visit http://ecampus.oregonstate.edu/phytophthora. English and Spanish language versions are available.

The *Phytophthora* Online Course: Training for Nursery Growers provides access to all levels of nursery personnel and the public worldwide. This free, non-credit course includes three modules: biology, symptoms, and diagnosis; disease management; and *Phytophthora ramorum*, the quarantine pathogen that causes sudden oak death in forest trees as well as ramorum blight on nursery plants. It takes about 4 hours to complete the course. For an optional $100 fee, nursery growers can earn a Certificate of Mastery after successfully completing an online exam. Those who pass the exam can also earn four pesticide recertification credits from the Oregon Department of Agriculture.

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Establishment of *Phytophthora ramorum* Depends on Woodland Diversity and Species Composition[1]

Nathan Rank,[2] Hall Cushman,[2] Ross Meentemeyer,[3] and David Rizzo[4]

Abstract

Recent ecological theory predicts that disease establishment depends on local diversity and on abundance of key vector species. These predictions have not been tested for plant pathogens in woodland environments. Here we investigate the relationship between woody species richness and the abundance of key tree species on establishment of *Phytophthora ramorum* on its main foliar host, California bay laurel (*Umbellularia californica*). We also document disease progression on bay laurel and coast live oak (*Quercus agrifolia*) over a 6-year period. Oak woodlands of coastal California occur across a range of topographic and environmental conditions that vary in local climate and support different species of woody plants. This variation in microclimate and vegetation type yields high variability in the likelihood of establishment of *P. ramorum* in California forests. For example, some areas support high densities of bay laurel, the most important foliar host of *P. ramorum*, while this host is rare in other areas. The risk of infection of coast live oak depends partly on the local abundance of bay laurel, which serves as a source of inoculum and transmission to canker hosts.

We analyzed plant community data in 200 randomly located 15 x 15 m plots in a 275 km^2 region in eastern Sonoma County, California. Within this network, bay laurel was the most widely distributed woody species, occurring in 97 percent of plots, followed by coast live oak (72 percent), Douglas-fir (*Pseudotsuga menziesii*) (47 percent), California black oak (*Q. kelloggii*), (45 percent), Pacific madrone (*Arbutus menziesii*) (43 percent), Oregon white oak (*Q. garryana*) (43 percent), and toyon (*Photinia arbutifolia*) (41 percent). When plots were established in 2003, we measured over and understory abundance of woody species and installed microclimate loggers to measure understory temperature and relative humidity. Since 2003, we have conducted annual surveys of forest structure for each main woody host of *P. ramorum*. In addition, we surveyed disease severity of *P. ramorum* from 2004 to 2009 through timed counts of symptomatic leaves on bay laurel. Using data from this plot network, we asked the following questions: 1) Does woody species richness affect pathogen prevalence on bay laurel hosts? 2) Does pathogen prevalence on bay laurel depend on the presence of coast live oak and black oak? 3) How does disease establishment on bay laurel, coast live oak, and black oak progress over time? 4) Does pathogen abundance on bay laurel relate to infection rate of coast live oak?

To assess the relationship between woodland species richness and *P. ramorum* establishment, we conducted multiple regressions that included number of species and bay laurel stem number as independent variables and either total number of bay laurel symptomatic leaves per plot or mean number of symptomatic leaves per bay laurel stem as dependent variables. The

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total number of leaves provided an index of inoculum load and the mean number of leaves per stem provided an index of disease prevalence. The multiple regressions revealed that pathogen abundance and prevalence declined with woodland species richness and increased with bay laurel abundance ($P < 0.005$ for both comparisons). This relationship suggests that species diversity acts as an encounter reduction mechanism with a dilution effect on disease risk.

Our results also showed that disease severity was lower in plots where coast live oak was present than in plots where it was absent (two-way factorial ANCOVA with bay laurel stem number as covariate; $P < 0.01$ for both comparisons). No such effect was observed for black oak. Bay laurel co-occurs with coast live oak more often than any other pair of woody species in our plot network (52 percent of plots) and the number of bay laurel stems per plot is negatively related to the number of coast live oak stems (Spearman's $r = -0.14$, $P < 0.05$). The effect of coast live oak on *P. ramorum* establishment suggests that pathogen transmission is reduced in areas where bay canopy is reduced by competition with coast live oak. Coast live oak seems to be one of the most important woodland species influencing pathogen establishment on bay laurel, and as the oak canopy declines due to disease progression, we may observe an increase in pathogen abundance and prevalence on bay laurel, which will eventually result in even greater levels of disease on coast live oak.

We also quantified disease progression on bay laurel and coast live oak from 2004 to 2009. From the outset, symptoms of *P. ramorum* have been observed on the vast majority of bay laurel stems (> 75 percent). Between 2005 and 2006, the number of symptomatic stems increased to over 90 percent of all bay laurel individuals, and it leveled off after 2007. In contrast, relatively few coast live oak or black oak stems show symptoms of *P. ramorum* infection (assessed by the presence of a canker on the trunk). In 2004, 3 percent of coast live oak stems possessed cankers, but the prevalence increased to 21 percent by 2009. The largest year to year increase in proportion of oak stems with cankers was observed from 2006 to 2007, suggesting a 1 year lag between the changes in the proportion of symptomatic bay stems and disease progression on oak. By 2009, we observed mortality of 5.8 percent of the total number of coast live oak stems ($n = 742$) after observation of a *P. ramorum* canker on the stem. This number represents a lower bound estimate of *P. ramorum*-related mortality.

Our data support the general hypothesis that oak mortality increases with increasing amounts of inoculum on leaves of bay laurel. The probability that a coast live oak stem possessed a canker by 2009 increased with increasing pathogen abundance (quantified by bay laurel symptomatic leaf number; logistic regression weighted by oak stem number; $n = 128$ plots, $P < 0.001$). We also observed that the probability of coast live oak mortality in a study plot by 2009 increased with increasing pathogen abundance on bay laurel (logistic regression weighted by oak stem number; $n = 128$ plots, $P < 0.001$). As coast live oak infection and mortality progress further, we may observe the same relationships between woodland species diversity and disease progression in coast live oak that we documented for bay laurel.

Using Rain Bucket Spore Traps to Monitor Spore Release During SOD Eradication Treatments in Oregon Tanoak Forests[1]

Paul Reeser,[2] Everett Hansen,[2] Wendy Sutton,[2] Alan Kanaskie,[3] Jon Laine,[3] Michael Thompson,[3] and Ellen Michaels Goheen[4]

Introduction

The complete Oregon eradication treatment for *Phytophthora ramorum*, the cause of sudden oak death, calls for early detection and: 1. prompt killing of infected trees plus a buffer of visibly healthy trees using herbicide when possible to prevent re-sprouting; 2. falling the killed trees and cutting other host plants; and 3. burning all slash. The goal is to halt sporulation and dispersal of sporangia as quickly as possible. These treatments are expensive, slow, and resisted by some landowners. There has been no rigorous comparison of the effectiveness of these tree killing methods on inoculum production, however. We report on trials to test these treatment variables, using baited rain traps to monitor production of sporangia under the different eradication treatment conditions.

Methods

Rain traps baited with rhododendron (*Rhododendron macrophyllum*) and tanoak (*Lithocarpus densiflorus*) leaves were placed beneath tanoak trees in areas distant from known infection, in known infested stands before eradication treatments began, and in infested stands during and after eradication treatments. Traps were moved as the treatments progressed, and traps were placed in new areas as *P. ramorum* was confirmed. Stands of green, apparently healthy tanoak were used as negative controls. Trapping continued for 18 months, ending April 2008.

Two-gallon white HDPE plastic buckets were lined with thin plastic bags. Bait leaves were placed in the bucket along with approximately 375 ml de-ionized water. The bucket was covered with a screen to keep out large debris. At retrieval (after 2 weeks of exposure), water depth was measured, the plastic liner replaced, and new bait leaves were added.

Bait leaves were kept in Ziploc® bags in a cooler and transported to the laboratory, where they were washed in tap water and blotted dry. Necrotic areas of leaves were plated in semi-selective CARP+ medium (Corn meal agar base amended with 30 ppm Benlate 75WP, 10 ppm Na-natamycin, 200 ppm Na-ampicillin, 10 ppm rifamycin

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SV, and 25 ppm hymexazol). Petiole ends were always included whether necrotic or not. Isolation plates were incubated in the dark at 20 ºC for 7 to 10 days, then examined for the growth of *P. ramorum*, which was recognized by a combination of distinctive hyphae, chlamydospores, and sporangia.

Trap placements were classified by site infestation status (infested or healthy), eradication treatment progress (untreated, partially treated, treatment completed), and canopy condition (infected tanoak, brush pile, non-host species, and others). There were usually 5, 10, or 15 traps placed at a site, depending on size of the tanoak stand or eradication area. Traps were scored as positive or negative for *P. ramorum* for each sampling interval based on culture results.

Results

Phytophthora ramorum inoculum is not generally distributed in the Curry County quarantine area. The pathogen was seldom recovered (1 percent of traps) from apparently healthy stands away from known infested areas. *P. ramorum* was recovered more often from traps placed inside the eradication area (29 percent of traps) than in traps placed in the eradication area perimeter, around 300 feet from the nearest infected tanoak (less than 1 percent of traps).

Recovery of *P. ramorum* was reduced in traps placed in infested stands during and after site treatment (table 1).

Table 1—Recovery of *P. ramorum* in infested stands

Site condition	Treatment status	Proportion traps positive for *P. ramorum*	Number of traps
Infested	None	0.60	447
Infested	Partial	0.27	301
Infested	Completed	0.11	1473
Healthy	None	0.01	343

In infested tanoak stands with active sporulation, the probability of detecting *P. ramorum* varied with canopy condition (table 2). Recovery of *P. ramorum* was reduced after application of the hack and squirt herbicide (imazapyr) treatment, but this effect was often months in occurring. Spores were recovered from trees with brown foliage due to hack and squirt on some occasions. In at least one location, *P. ramorum* was detected in open ground at a fully treated site 10 months after all tanoak canopy had been removed, piled, and burned. This may have been due to a blow-in event or rain splash from the forest floor.

Table 2—Recovery of *P. ramorum* in relation to crown condition

Canopy condition in eradication area	Proportion traps positive for *P. ramorum*	Number of traps
Symptomless Tanoak	0.31	71
Infected Tanoak	0.70	371
Tanoak - Herbicide	0.53	156
Myrtlewood	0.41	129
Brush Pile	0.21	189
Non-Tanoak Species	0.15	185
Alder	0.05	86
Rhododendron	0.0	23
Huckleberry	0.0	25
Open Ground	0.48	27

In infested stands, *P. ramorum* was recovered at a high frequency, apparently correlated with rainfall, as measured by water depth in the rain trap at the time of retrieval (fig. 1). In rainy periods, the pathogen was recovered from 60 to 95 percent of the rain traps. This effect may be confounded with season and changing site characteristics as eradication proceeded.

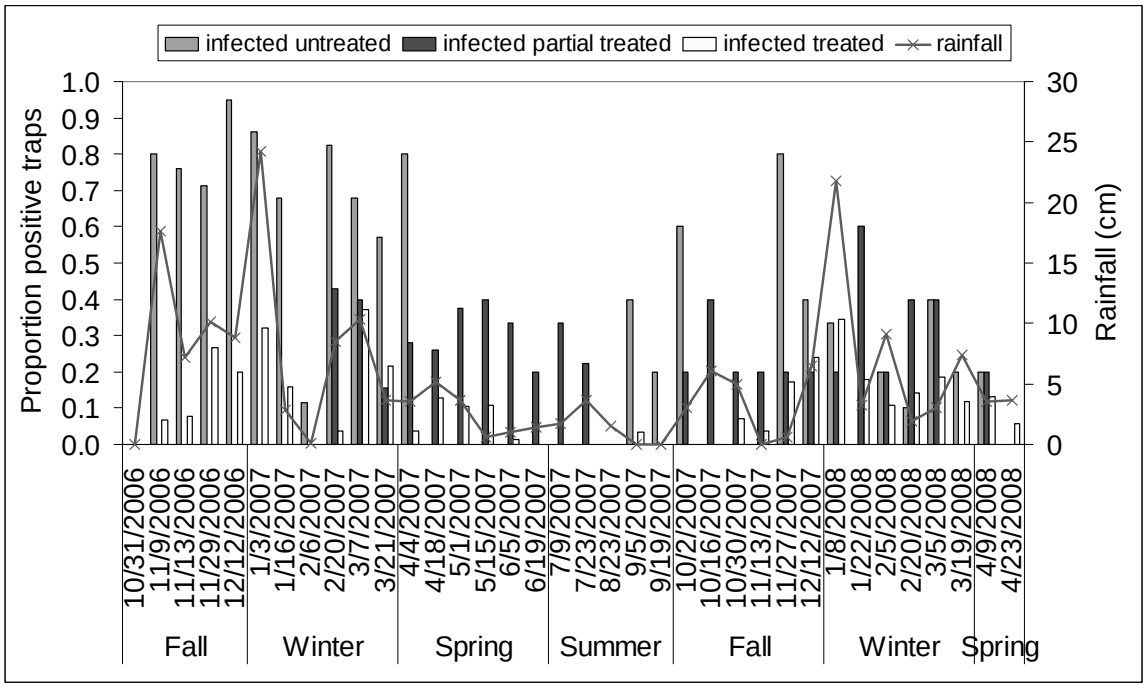

Figure 1—Inoculum was available during rain events throughout the year. *P. ramorum* was recovered from bait leaves in rain traps in 34 of 36 2-week sample periods. Periods with reduced rainfall (Red Mound Remote Automated Weather Station, http://www.wrcc.dri.edu/cgi-bin/rawMAIN.pl?orOREM) tended to have reduced detection of *P. ramorum*.

Summary

1. Inoculum is available during rain events throughout the year. *P. ramorum* was recovered from bait leaves in rain traps in 34 of 36 2-week sample periods.

2. *Phytophthora ramorum* inoculum is not generally distributed in the Curry County quarantine area. *P. ramorum* was seldom recovered from apparently healthy stands away from known infested areas.
3. In infested stands, *P. ramorum* was recovered at a high frequency, apparently correlated with rainfall. In rainy periods, the pathogen was recovered from 60 to 95 percent of the rain traps.
4 Recovery in infested stands during and after site treatment was reduced and the pathogen was never recovered from traps placed just outside the treatment areas.

A second trial is underway, with buckets placed beneath individual trees of known infection and treatment status. This will allow us to separate stages of the treatment process and better assess which treatments have the biggest impact on inoculum production.

Acknowledgments

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Chip-Based On-Site Diagnosis of *Phytophthora ramorum*[1]

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Abstract

The pathogen *Phytophthora ramorum* Werres, De Cock, & Man in't Veld which attacks a wide range of host plants and is a worldwide threat to natural ecosystems and the nursery industry. To prevent the spread of this pathogen with latently infected plants, easy to handle diagnostic systems with high sensitivity and high throughput are demanded. Furthermore, such methods should give results within a short time on site of inspection. A 3-year project is underway to create a chip-formatted PCR system combined with a chip-based electrical microarray that will be adapted for the detection of *P. ramorum* in plant tissue. A stationary PCR chip with integrated microstructured heaters and temperature sensors has been developed for the amplification of specific *Phytophthora* DNA fragments. A microfluidic system connects the PCR chip with a microarray where the labelled DNA fragments are detected. The miniaturization of the chip formatted PCR and array system enables high portability, low input of energy, smaller amounts of expensive analytic chemicals and due to low reaction volumes very fast reaction times.

Introduction

Determination of *Phytophthora ramorum* in latently infected plants or in plants with unspecific symptoms presupposes a (mostly centralized) microbiological laboratory, experienced personnel, and time. But, to prevent the spread of *P. ramorum* with latently infected plants, decisions in phytosanitary management have to be made fast. Therefore, easy to handle diagnostic systems with high throughput and high sensitivity are in need.

Here we present the development of a portable chip-based nucleic acid detection system for the identification of *P. ramorum* and other commercially important *Phytophthora* species. This system will enable the fast and specific diagnosis of a great number of samples in the field.

Material and Methods

For the demonstration of the detection system, the ITS2 area of the ribosomal DNA was chosen as the target sequence for the identification of *Phytophthora* species. For many *Phytophthora* species, this region is sufficient for discrimination. To optimize the chip-formatted reactions, both parts of the detection process (PCR and hybridization) were developed in separate approaches.

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[1] A version of this paper was presented at the Fourth Sudden Oak Death Science Symposium, June 15-18, 2009, Santa Cruz, California.

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PCR-Chip Module

A stationary PCR chip (fig. 1A) was developed which integrates microstructured thin heater structures and temperature sensors. The PCR reaction is performed in a PDMS (Polydimethylsiloxane) reaction chamber on top of the stationary PCR chip. The reaction chamber is disposable (one time usage) so that any cross contaminations can be prevented. The PCR protocol is designed as a two-step protocol (combined annealing and elongation step). After PCR, the reaction products are transported to the detection chip by an automatic microfluidic system.

DNA-Chip Module

The PCR products are analyzed with a chip formatted microarray (fig. 1B). This disposable chip combines a microfluidic hybridization chamber with electrode structures. The hybridization is designed as flow through hybridization. DNA probes are spotted at the electrode gap. Detection of the hybridization with species-specific probes is performed by enzymatic silver enhancement. Successful hybridization results in an aggregation of silver particles at the electrode gap. The resulting change of the electrical resistance is measured. The hybridization can also be analyzed optically by measuring the gray scale value of the silver aggregation at the spotting point.

Both chips are combined in a united platform for the amplification, labelling, and qualitative detection of *Phytophthora* DNA. The software for control maintenance and analysis of the reactions can be run on an average Personal Digital Assistant (PDA).

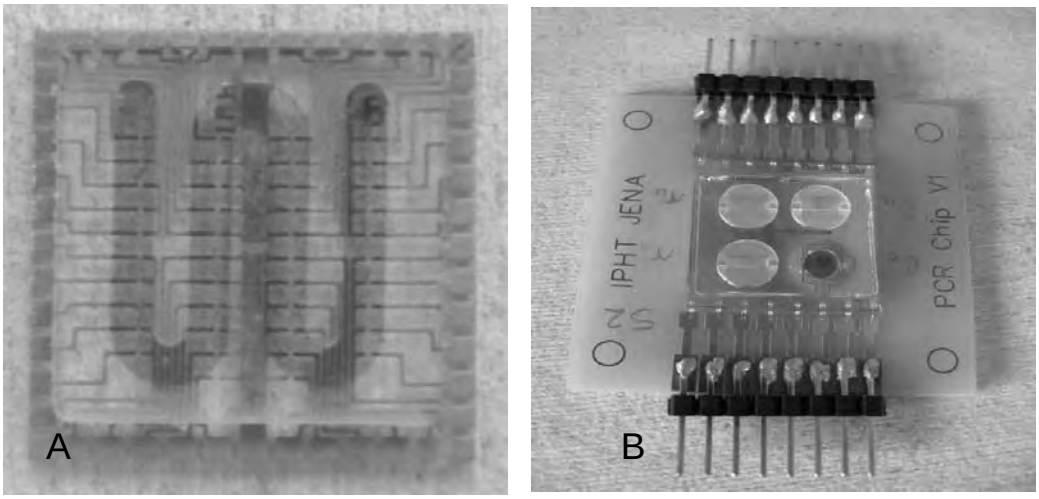

Figure 1—Stationary PCR chip with four integrated microstructured heaters, temperature sensors, and PDMS chamber for on-chip amplification and labeling of DNA (A); gold microarray chip combined with a microfluidic PDMS channel (prototype), specific oligonucleotide probes are spotted at 42 detection sites (B).

Preliminary Results

PCR protocols for chip-based amplification of the approximately 550bp ITS2 sequences were adapted and optimized. Amplification products produced with the

PCR chip are identical with PCR products from conventional thermocyclers (fig. 2). The two-step PCR protocol helped to reduce the amplification time by more than 50 percent. Advantages of on-chip amplification are very short heating and cooling times and reduced input of chemicals and energy. These advantages can be achieved by the low reaction volume (up to 0.5 µl) of the chip PCR.

For the identification of *P. ramorum*, different species-specific probes (30 basepairs) located at the ITS2 region were developed and tested in hybridization assays. The specificity of the *P. ramorum* probes was tested with a range of closely related *Phytophthora* species including *P. lateralis* and *P. hibernalis* (fig. 3). The flow through hybridization in the microfluidic hybridization chamber has the advantage of reducing the reaction time and the reagent volumes.

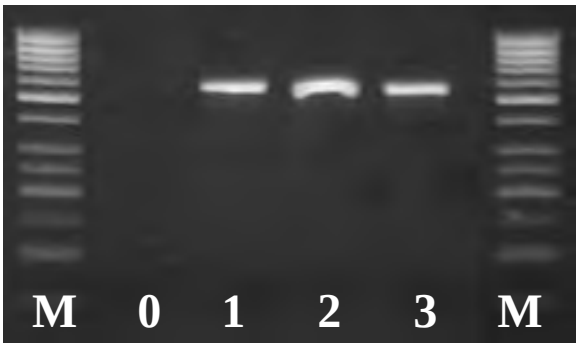

Figure 2—PCR chip: amplification of the *P. ramorum* ITS2 fragment.
0: negative control (buffer);
1: PCR in conventional thermocycler;
2: PCR in PDMS chamber chip;
3: PCR on glass chip;
M: DNA size marker.

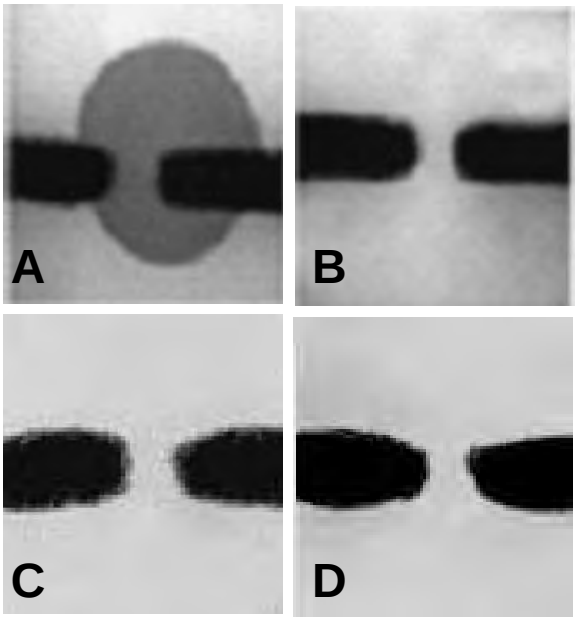

Figure 3—DNA chip: hybridization of *P. ramorum*-specific probe with ITS2 PCR products (ca 550bp) of *P. ramorum* and closely related *Phytophthora* taxa *P. lateralis* and *P. hibernalis* (optical analysis of gray scale value of the aggregated silver nanoparticles at electrode gap).
A: specific hybridization: *P. ramorum* x *P. ramorum*;
B: unspecific hybridization: *P. ramorum* x *P. lateralis*;
C: unspecific hybridization: *P. ramorum* x *P. hibernalis*;
D: negative control *P. ramorum* x buffer.

Acknowledgments

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Burn it, Chip it, or Tarp it, but Just Don't Move it: Managing Oak Firewood Infested With the Goldspotted Oak Borer, *Agrilus coxalis auroguttatus*[1]

Steven J. Seybold,[2] Tom W. Coleman,[3] and Mary Louise Flint[4]

Abstract

In June 2008, a new and potentially devastating pest of oaks, *Quercus* spp., was discovered in San Diego County, California. This pest, the goldspotted oak borer (GSOB), *Agrilus coxalis auroguttatus* Schaeffer (Coleoptera: Buprestidae), colonizes the sapwood surface and phloem of the bole and larger branches of at least three species of *Quercus*. Larval feeding kills patches and strips of the phloem and cambium resulting in crown dieback followed by mortality. Since 2002, aerial surveys in San Diego County have detected about 20,000 dead oaks. In a survey of forest stand conditions at three sites in this area, 67 percent of the oaks had external or internal evidence of GSOB attack. The damage is worst for mid-story and massive overstory oaks in the red oak group (subgenus *Quercus*, section *Lobatae*), which are succumbing to colonization at a rate of 90% in some areas. In the worst cases, up to 50% of oaks >12 cm are dying. Because of its recent discovery in California, specific management practices for GSOB are still being tested. However, given its potential for damage, landscape and land managers need guidelines for managing this pest now. We are beginning to develop the first components of an integrated pest management (IPM) program for this new pest based on IPM principles developed for other well-known *Agrilus* spp. pests of shade trees, for example, the bronze birch borer, *A. anxius*, and the emerald ash borer, *A. planipennis*.

Key techniques in the plan include monitoring the adult flight period and sanitation of oak firewood by various methods including solarization. Determining the flight period and enhancing survey techniques will be crucial for managing GSOB populations and preventing oak mortality. In 2008, purple and lime green flight-intercept panel traps were found to be effective for trapping GSOB. We assessed the flight period from June to November in 2008 and resumed trapping in January 2009. Various lures (manuka oil, phoebe oil, and ethanol) and trap placements were also assessed in 2009 to improve efficacy of trap catch and detection.

Movement of firewood is a major pathway of dispersal for many insect pests in the U.S., and represents one hypothesis for the introduction of GSOB into California. Outreach efforts to minimize further movement of infested firewood within the state and to encourage treatment of firewood are also essential. We assessed three solarization treatments of firewood: 1) direct sun exposure; 2) tarping with clear plastic and sun exposure; and 3) caging of firewood and storage in a shaded area to monitor normal woodborer emergence (control treatment). These treatments were replicated at two elevations (1,220 and 1,740 m) at sites that include recently

[1] A version of this poster was presented at the Fourth Sudden Oak Death Science Symposium, June 15-18, 2009, Santa Cruz, California.

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GSOB-killed coast live oaks (*Quercus agrifolia*) and California black oaks (*Q. kelloggii*), respectively. We are also looking at the efficacy of chipping firewood.

This study will develop methods for reducing GSOB populations and oak mortality. Educational materials from this study will be widely disseminated to the public to reduce the movement of untreated firewood and slow the spread of GSOB throughout California.

Introduction

The goldspotted oak borer (GSOB), *Agrilus coxalis auroguttatus* Schaeffer (Coleoptera: Buprestidae), is a new threat to oaks in southern California. Tree mortality has been recorded since 2002, but was attributed to drought for many years. This phloem/wood borer was first collected in San Diego County in 2004, but not linked to the on-going tree mortality until June 2008 (Coleman and Seybold 2008a). We hypothesize that GSOB was most likely introduced on firewood from its native distribution in Arizona and Mexico. There are no collection records of GSOB from localities between the native habitat and California, ruling out a natural range expansion for GSOB (Coleman and Seybold 2009, 2010).

Aerial surveys of San Diego County have detected an estimated 20,000 dead oaks over the past 8 years. In this area, GSOB injures and kills coast live oak (*Quercus agrifolia*), California black oak (*Q. kelloggii*), and canyon live oak (*Q. chrysolepis*). There is no previous record of injury or mortality to oaks from GSOB in Arizona and Mexico. This species has been rarely collected in its native region and very little was known about its life history and biology prior to 2008.

Signs of GSOB injury include thinning crowns, black staining of the outer bark primarily along the bole, bark removal by woodpecker foraging, and D-shaped adult exit holes (Coleman and Seybold 2008b). Repeated larval feeding at the interface of the phloem and xylem kills patches of the cambium, which leads to tree mortality after several years.

This new pest is killing high-value landscape and shade trees, impacting aesthetic beauty of urban-wildland landscapes and property values. Loss of a single shade tree or the cost of removing hazardous trees around dwellings entails significant costs (upwards to $50,000). We describe a provisionary plan for an integrated pest management (IPM) program for this new pest based on IPM principles developed for other well-known *Agrilus* spp. pests of shade trees.

Key techniques in the plan include monitoring adult flight period and sanitation of oak firewood by various methods including solarization. We focussed on three main objectives for adult trapping: 1) determine the adult flight period with purple and lime-green prism flight-intercept sticky panel traps; 2) determine the most effective height for purple and lime-green prism traps; and 3) assess lures for enhancing adult trap catch.

Movement of firewood is a major pathway of dispersal for many insect pests in the U.S., and represents the primary hypothesis for the introduction of GSOB into California. There is evidence of previous and current movement of firewood into and out of the infested area of San Diego County. Outreach efforts to minimize further movement of infested firewood within the state are also essential. We assessed three

solarization treatments of firewood: 1) direct sun exposure; 2) tarping with clear plastic and sun exposure; and 3) caging of firewood and storage in a shaded area to monitor normal woodborer emergence (control treatment).

Methods and Results

Adult Trapping

In 2008, purple prism flight-intercept panel traps were established in late June in two forest stands of coast live oak (four traps/site). Maximum flight was recorded during the first week that the traps were in place and flight declined until it ceased in November (Coleman and Seybold 2008a).

In January 2009, this work was continued with both purple and lime-green traps, but was expanded to two elevations (1,035 and 1,765 m), which also represent two distinct host distributions (coast live oak and California black oak). Three sites were established at each elevation and three traps of each color were placed at each site. The traps were placed at three heights: low (1.5 m), medium (3 m), and high (4.6 m). Traps were monitored weekly until mid-September.

In 2009, GSOB did not begin flying until the week of May 15 to 22 (lower elevation site). GSOB flight did not begin until the week of May 29 to June 5 at the high elevation site. Peak flight activity occurred between mid-June and early July at both sites. Flight activity declined rapidly in early August; beetles were still trapped in mid-September, but the numbers were very low. Purple prism traps were more effective at catching GSOB than lime-green traps, but the difference was not significant. Purple prism traps were most effective when hung at 3.0 m above ground, whereas lime-green traps were most effective when hung at 4.5 m above ground.

From June to August 2009, four lures were assessed to enhance trap catch: 1) manuka oil; 2) phoebe oil; 3) (*Z*)-3-hexenol; and 4) no lure. Twenty-four traps were used across three sites (four green and four purple/site). Manuka oil is a steam distillate of the New Zealand manuka tea tree, *Leptospermum scoparium*, whereas phoebe oil is a steam distillate of Brazilian walnut, *Phoebe porosa*. None of the trap baits were effective at attracting GSOB.

Firewood

Firewood pieces (~30 cm long x 10-20 cm dia.) from recently killed (<1 yr) coast live oak and California black oak were collected in the winter of 2008-2009 from >15 trees and the pieces were randomized within species prior to assignment to three treatment groups (i.e., six treatments total). The experiment was established in February 2009 and duplicated at two sites: Low (1,220 m) and high (1,740) elevations. Treatment replicates consisted of six to eight pieces of wood that were caged in aluminum mesh screening. The three treatment groups were: 1) direct sun exposure; 2) tarping with clear plastic and sun exposure; and 3) caging of firewood and storage in a shaded area to monitor normal woodborer emergence (control treatment). For the tarping treatment, two layers of thick, 8 mil clear plastic were used. The tarp edges were covered with soil to prevent beetles from escaping and to

trap heat beneath the plastic. All treatments were sampled weekly for GSOB emergence until September. HOBO data loggers recorded temperature and relative humidity in selected replicates from all treatment groups. Mean GSOB emergence pooled over oak species and elevation was the primary variable that we measured (N=36). Treatments were analyzed statistically by using a mixed model of analysis of variance.

Adult GSOB were recovered from firewood of both species of oaks. Adult emergence began the week of May 22 and continued into July and August. No significant differences were detected among the three treatments. Initial beetle emergence occurred two weeks earlier in the direct sun exposure and tarping treatments, most likely due to the increased temperatures in the sun-exposed wood piles. However, GSOB emergence was significantly greater in piles of coast live oak than in piles of California black oak.

Temperature and relative humidity were highest in tarped treatments when measured over the duration of the study; the temperatures under the tarp reached 52.4 °C during daytime highs. Temperatures were also slightly higher in direct sunlight treatments than in shaded controls.

Discussion

Our capability for trapping GSOB adults is in its infancy. Purple-colored traps were slightly more effective than lime-green-colored traps and a height of 3 m (relative to 1.5 or 4.6 m) was the most effective. None of the bait combinations that we tested seemed to enhance trap catch and much more needs to be done to identify specific host- or beetle-produced attractants for GSOB. The flight of GSOB appears to begin in mid-May and then peak in mid-June to early July (Coleman and Seybold 2008a, this study). Flight declines rapidly in August and then continues at a very low level into the fall. Additional flight trapping data will allow us to better understand the biology and phenology of this new pest. This information will be critical to effectively time preventive pest management practices including insecticide applications when they are necessary.

We reared GSOB in spring/summer 2009 in the field from firewood pieces that had been cut from trees that died in 2008. New adult beetles emerged from this wood from mid-May through mid-summer. Thus, the immature and mature stages of the beetle can survive in small pieces of cut wood through the fall and winter, even under harsh treatment conditions. This underscores and justifies the concern of moving and properly managing firewood. Also this supports the hypothesis that GSOB was introduced into California in firewood. Proper firewood management will potentially slow the spread of this insect from San Diego County into other parts of California.

Although the direct sunlight and tarping treatments did not suppress GSOB emergence relative to the shaded control, tarping firewood will likely prevent adults from escaping. Future studies (2010) will assess other types of plastic, the capacity of plastic to trap emerging beetles, and the effect of chipping as an option for managing infested firewood. Chipping infested wood may be the best method for reducing GSOB population density, and this technique has been worked out recently for the emerald ash borer, *A. planipennis*, in the eastern U.S. Treating firewood may

reduce localized beetle populations, which can protect adjacent high-value shade trees.

Acknowledgments

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Stream Monitoring for Detection of *Phytophthora ramorum* in Oregon Tanoak Forests[1]

Wendy Sutton,[2] Everett Hansen,[2] Paul Reeser,[2] Alan Kanaskie,[3] and Harvey Timeus[3]

Abstract

Stream monitoring using leaf baits for early detection of *Phytophthora ramorum* is an important part of the Oregon sudden oak death (SOD) program. Fifty-eight streams in and near the Oregon quarantine area in the southwest corner of the state are currently monitored. The watersheds monitored range in size from 8 to 3592 ha, with a combined area of 323 656 ha. (fig. 1).

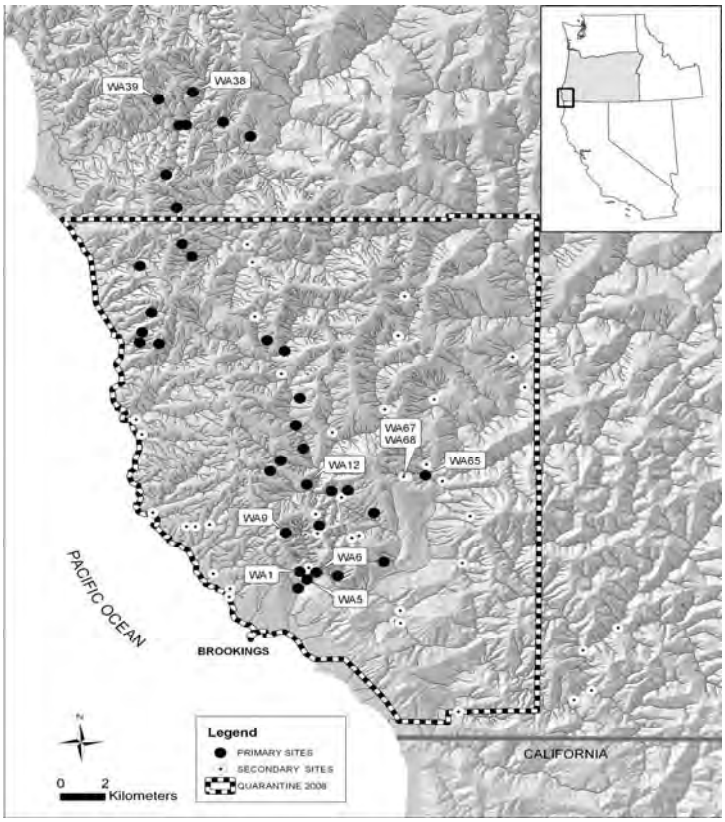

Figure 1—Map of southwest Oregon shows current quarantine area. Primary sites have been continuously sampled over time; secondary sites have been sampled varying lengths of time.

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Rhododendron (*Rhododendron macrophyllum*) and tanoak (*Lithocarpus densiflorus*) leaf baits in mesh bags are exchanged every 2 weeks throughout the year.

Early in the monitoring all samples were assayed by multiplex ITS PCR and isolation on selective medium. The correlation between the two tests is strong and eventually only PCR was performed (table 1). If a previously negative site assayed positive for *P. ramorum*, the sample was plated on selective medium.

Table 1—Correspondence between culture and multiplex PCR results for detection of *P. ramorum* in 1,804 stream bait samples collected from 2003 through 2007 and tested by both methods

Diagnostic Method		
Culture	**PCR**	**Percent of Samples**
Positive	Positive	5.3
Positive	Negative	0.8
Negative	Positive	11.8
Negative	Negative	82.2

Once a site tested positive for *P. ramorum,* follow-up intensive ground surveys located infected tanoaks or other host plants an average of 295 m upstream from the bait station. Stream baits have been positive for *P. ramorum* up to 1150 m (stream distance) from the probable inoculum source.

In eight watersheds *P. ramorum* was detected in stream baits before we found infected plants. An example is stream WA65 which drains an old-growth redwood stand on 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; all host plants on the sites were cut and burned later that year.

Phytophthora ramorum was detected by both culturing and multiplex PCR of leaf baits in all seasons of the year. Sampling was sometimes impossible in late fall and winter due to fluctuating water levels associated with winter storms. In streams with lower frequency of recovery of *P. ramorum*, a seasonal trend was evident. Detection of *P. ramorum* was usually lowest from December through April, with peak recovery rates from July through October (fig. 2).

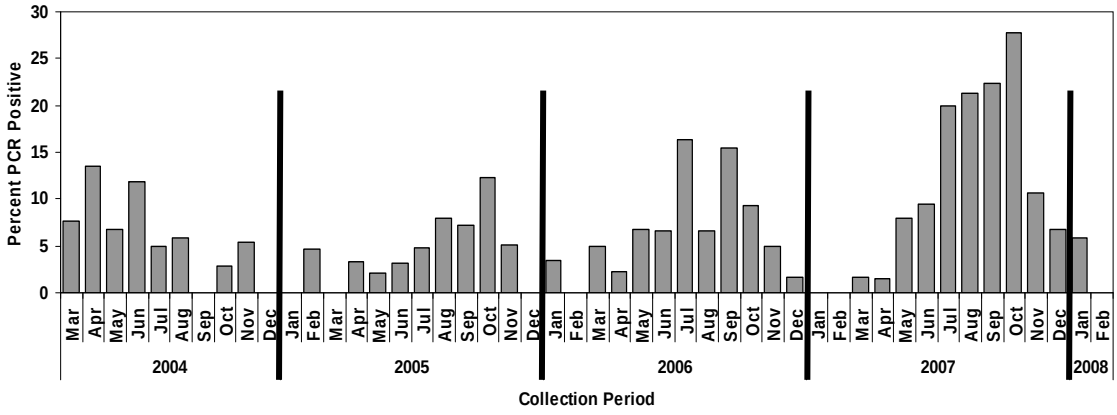

Figure 2—Detection of *P. ramorum* by multiplex PCR from leaf baits in 32 streams monitored continuously from March 2004 through February 2008. There were no samples collected in September 2004.

Periods of low recovery frequency corresponded with cooler water temperatures (fig. 3) and high winter flow rates in the streams (data not shown).

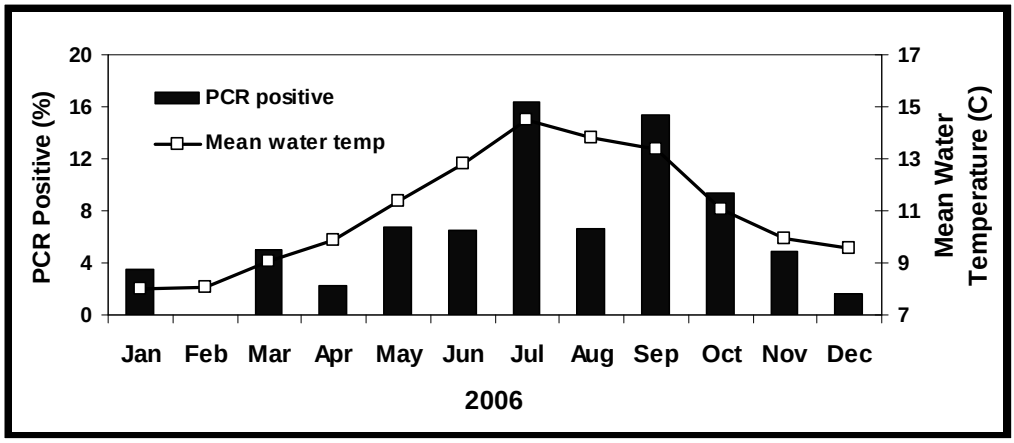

Figure 3—Monthly multiplex PCR detection of *P. ramorum* in leaf baits from 32 streams monitored in 2006 and water temperature of those streams.

Acknowledgments

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Factors Affecting Onset of Sporulation of *Phytophthora ramorum* on Rhododendron 'Cunningham's White'[1]

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

Abstract

Phytophthora ramorum is the Oomycete pathogen that causes sudden oak death (SOD). SOD continues to cause heavy losses to California and Oregon forests as well as to the U.S. nursery industry (Dart and Chastagner 2007; Frankel 2008), in part due to quarantine regulations. Epidemics are driven by sporangia, which are produced in large numbers on various *P. ramorum* hosts (Parke and others 2002; Tooley and Browning 2009). While some sporulation has been observed on leaves of coast live oak (*Quercus agrifolia*) (Vettraino and others 2008), California bay laurel (*Umbellularia californica*) is thought to be the major producer of sporangia that drive forest epidemics (Davidson and others 2008). Little is known of the parameters associated with the onset of sporangia production by *P. ramorum* on its hosts following infection and on leaves with established disease. We conducted experiments to examine the relationship between lesion size, moisture period, and temperature with the first appearance of dehisced sporangia. Leaves exhibiting small lesions induced via artificial inoculation with isolate CAM 5C using a variety of techniques were positioned on 15 µm-mesh nylon screens in a mist chamber inside a greenhouse cubicle (15 to 18 °C). Lesions were measured daily, and sporangia were collected and counted. Lesion size did not appear to be a reliable predictor for onset of sporangia production. We observed a wide range of lesion sizes (1 to 54 mm^2) corresponding to the initial collection of dehisced sporangia from diseased leaves. In contrast, the longer the moist period duration, the higher the percentage of diseased leaves supporting sporangia production by *P. ramorum* – 17 to 22 percent within 24 hours, and an additional 39 to 64 percent within 48 hours. On leaves with established disease, dehisced sporangia were collected from ≥ 50 percent of leaves within a 6- to 12-hour moist period. In temperature studies, sporangia were collected from misted detached leaves within 1 day at 15 °C; 2 days at 10 °C and 20 °C; and 3 days at 4 °C, 25 °C, and 30 °C. When infected leaves were first pre-incubated at 20 °C for 72 hours, allowing lesions to expand uniformly, sporangia were collected from some leaves following a 1 day incubation at all temperatures. Knowledge of the timing and conditions that allow *P. ramorum* to sporulate following host infection will contribute to our understanding of epidemic dynamics and improve our ability to predict the likelihood that *P. ramorum* may spread within forest and nursery environments (Moralejo and others 2006).

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Screening *Trichoderma asperellum* as a Mycoparasite on *Phytophthora ramorum*[1]

Timothy L. Widmer[2] and Gary J. Samuels[3]

Abstract

Despite efforts of eradication and sanitation, *Phytophthora ramorum* persists in the United States and abroad. Fungicides have limited effectiveness, but there are concerns that they may only inhibit pathogen growth and resistance may develop. Biological control is an active control measure that can work continuously as long as the agent is alive and active. The goal of this study was to examine whether *Trichoderma asperellum* isolates are mycoparasitic on *P. ramorum*. Sixteen isolates of *T. asperellum* and other *Trichoderma* spp. that have demonstrated antagonism towards other *Phytophthora* spp., or were suspected to have mycoparasitic activity, were selected. The rate of mycoparasitism was determined by overlaying a strip of *Trichoderma*-colonized agar on a V8 agar plate colonized by *P. ramorum* (A2 mating type). Every 7 days for 4 weeks agar plugs were removed and transferred to V8 agar amended with benomyl (V8+B) or a wounded leaf disk of *Rhododendron* 'Cunningham's White.' Control plugs of *P. ramorum*, without exposure to the *Trichoderma* spp., always showed growth on V8+B and produced necrosis on the leaf disks. The different *Trichoderma* spp. isolates demonstrated variable mycoparasitic activities. Some isolates showed no inhibition of *P. ramorum* growth on V8+B or reduction in necrosis even from plugs removed directly below the *Trichoderma* strip. Other isolates showed a reduction in growth and necrosis over time, but did not completely eliminate the pathogen after 4 weeks. Six isolates of *T. asperellum* were consistent among replicated trials in eliminating growth of *P. ramorum* from the agar plugs and preventing leaf disk necrosis within 2 weeks exposure. Further testing of four of these six *T. asperellum* isolates against two different *P. ramorum* isolates (A1 and A2 mating types) resulted in the same high level of mycoparasitic activity. We believe the results demonstrate that specific *T. asperellum* isolates have the potential to remediate *P. ramorum*-infested soil. Tests are ongoing to determine whether *P. ramorum* soil populations can be eliminated when treated with these isolates.

Introduction

Studies have shown that *Phytophthora ramorum* can survive in potting medium around containers of infected plants in a nursery (Jeffers 2005, Tjosvold and others 2009). Aerated steam and chemical fumigants are known methods to eliminate soilborne pathogens. Linderman and Davis (2008) found that *P. ramorum* populations in potting media were killed by aerated steam heat treatments of 50 ^{o}C or higher or treatment with metam sodium concentrations of 0.25 ml per l of medium. However, using these techniques increases the chance of destroying beneficial microorganisms and of working in a hazardous environment.

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[1] A version of this paper was presented at the Fourth Sudden Oak Death Science Symposium, June 15-18, 2009, Santa Cruz, California.

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Some of the most studied and promising fungi used in a biocontrol system are *Trichoderma* spp. (Harman and others 2004). Populations of *Trichoderma* spp., which often are abundant in composts and compost-amended media, typically suppress *Pythium* and *Phytophthora* root rots within days after their formulation (De Ceuster and Hoitink 1999). *Trichoderma* spp. are reported to suppress soilborne diseases caused by *Phytophthora* spp. in containerized systems (da S. Costa and others 2000, Sharifi Tehrani and Nazari 2004). Currently, *T. asperellum* is being studied as a biological control agent to manage black pod disease of cacao in Cameroon. Recent results show that disease incidence was lower when *T. asperellum* was applied on infected cacao trees (Tondje and others 2007). It was the purpose of this study to screen selected *Trichoderma* spp. for antagonism towards *P. ramorum*.

Materials and Methods

Sixteen different *Trichoderma* spp. isolates were cultured on half-strength PDA (1/2PDA). This included 12 isolates of *T. asperellum*, two isolates of *T. virens*, one isolate of *T. koningiopsis*, and one undescribed isolate *Trichoderma* sp. nov. Three different *P. ramorum* isolates, WSDA-1772, 5-C, both A2 mating type and clonal lineage NA1, and PRN-1 (CBS 101327), mating type A1 and clonal lineage EU1, were cultured on 20 percent clarified V8 agar.

An agar plate bioassay was conducted as described by Krauss and others (1998). The *Trichoderma* spp. were grown on 1/2PDA in 90 mm diameter Petri plates until they completely colonized the plate. A 4 X 1 cm strip of *Trichoderma*-colonized 1/2PDA was removed and transferred to a *P. ramorum*-colonized V8 agar plate. A non-colonized 1/2PDA strip was used in the same way as a control. Every week for 4 weeks a 1 cm X 4.5 cm strip perpendicular to the original *Trichoderma* strip was removed. The strip was cut lengthwise in half and divided into 0.5 cm cubes to give two sets of nine cubes. From one of the sets the cubes were placed individually on the abaxial side of nine wounded *Rhododendron* 'Cunningham's White' leaf disks (6 mm diameter). The corresponding set was placed on a 20 percent V8 agar plate supplemented with 50 mg/l of benomyl. After 1 week at 20°C, observations were made on the leaf disks for necrosis and mycelial growth originating from the cubes on the V8 agar plate. The experiment was conducted twice for each *Trichoderma* isolate on the three different *P. ramorum* isolates.

Results

There was an observed correlation between the lack of necrosis on the leaf disks and no mycelial growth from the corresponding plug on the agar plate. Nine *Trichoderma* spp. isolates showed some reduction in necrosis after 1 week and complete reduction in necrosis and *P. ramorum* growth within 2 weeks after exposure. Eight of these isolates were *T. asperellum* and the other one was *T. koningiopsis*. There was no difference among the *P. ramorum* isolates tested.

Microscopic examination of the interaction between the antagonistic *Trichoderma* spp. and *P. ramorum* revealed mycoparasitism of *P. ramorum* chlamydospores and sporangia (fig. 1). This confirms the observation by Watanabe and others (2007), who first reported mycoparasitism as the mode of action of *T. asperellum*.

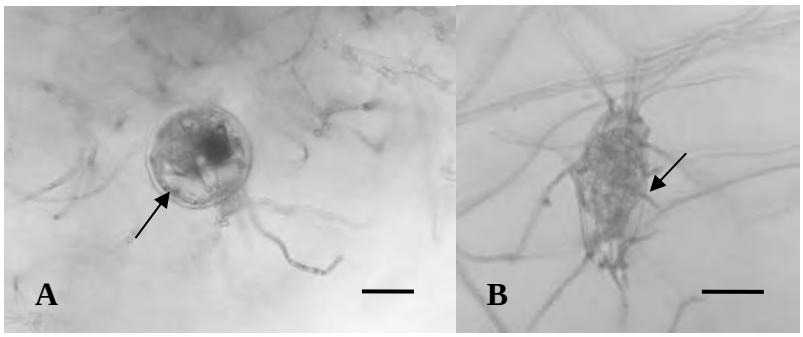

Figure 1—*Trichoderma asperellum* (arrows) demonstrating mycoparasitism of a *P. ramorum* **A)** chlamydospore and **B)** sporangium. Bar = 20 µm.

Discussion

Specific *T. asperellum* and *T. koningiopsis* isolates have potential as biocontrol agents against *P. ramorum*. Further tests have been started to determine if selected isolates can reduce *P. ramorum* soil populations to nondetectable levels over time. In addition, tests are ongoing to determine if selected isolates can parasitize and eliminate viability of *P. ramorum* propagules in infested leaf litter. If the *Trichoderma* spp. are found to be effective in eliminating the population of *P. ramorum* in the soil and infested leaf litter, then the potential exists to use this as a biologically based method to remediate infested nursery beds.

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