

Posters



Status of California's *Phytophthora ramorum* Stream Survey¹

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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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Sampling Cankers and Lesions on Eastern Forest Plant Species for *Phytophthora* spp. and Associated Organisms¹

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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	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>	-	-	-
	Coonskin SP	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>	-	-	-
	Beech Fork SP	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>	-	-
	Camp Creek SP	<i>P. cinnamomi</i> , <i>P. quercetorum</i>	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>	-	-
	Chestnut Ridge Park	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>	-	-	-
	Cacapon SP	<i>P. quercetorum</i>	-	-	-	-
	Buchanon SP I	<i>P. cinnamomi</i> , <i>P. cambivora</i> , <i>P. quercetorum</i>	<i>P. cinnamomi</i> , <i>P. cambivora</i>	-	-	-
		<i>P. europaea</i> , <i>P. cambivora</i>	-	-	-	-
PA	Buchanon SP II	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>	-	-
OH	Scito Trail SF I	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>	-	-
	Scito Trail SF II	<i>P. cinnamomi</i>	<i>P. cinnamomi</i>			

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An Evaluation of *Phytophthora ramorum* Preventative Treatments for Nursery and Forest Understory Plants in British Columbia, Canada¹

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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.	<i>Streptomyces lydicus</i>	0.9g/litre	901ppm + 156ppm Silwet
Rhapsody® ASO, Agraquest	<i>Bacillus subtilis</i>	20ml/litre	20,000
Sonata® ASO, Agraquest	<i>Bacillus pumilus</i>	10ml/litre	10,000
Actigard®, Syngenta	benzothiodiazole	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.

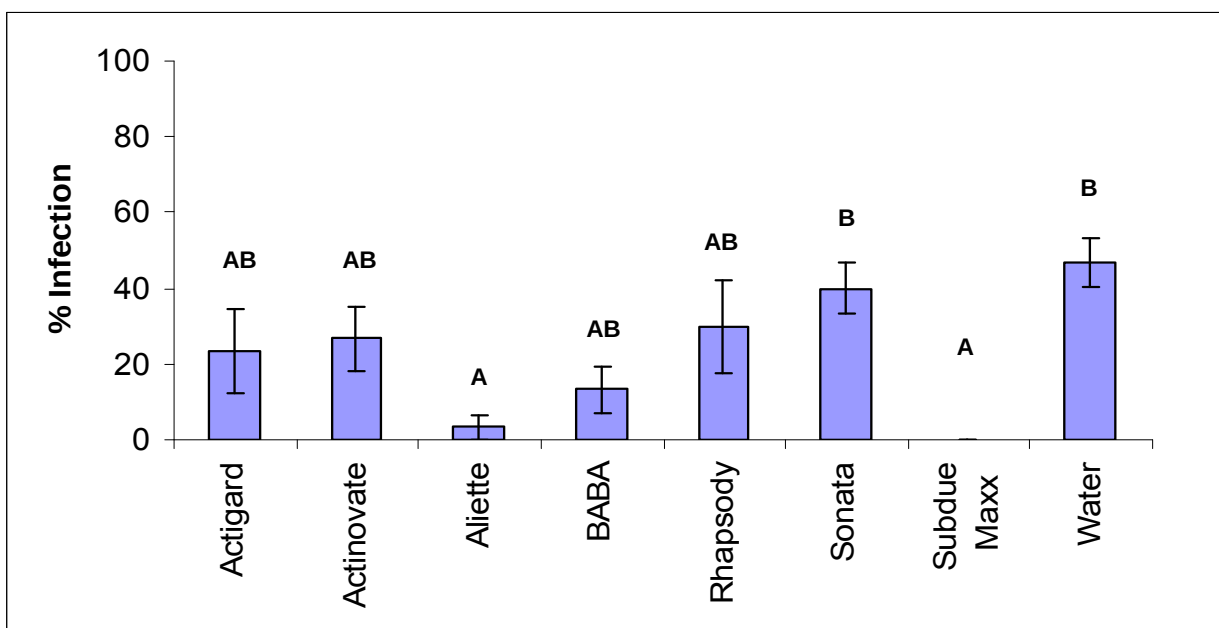


Figure 1—Disease response on excised leaves of treated plants. The number of leaves with lesions is given as a percent of total, per treatment. A one-way ANOVA comparing treatment means was performed. Treatments with the same letter were not significantly different according to the Tukey test at $P = 0.05$.

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 2008¹**

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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 Death¹

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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 Tanoaks¹

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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 than 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 than 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 than 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.

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Waiting for SOD: Sudden Oak Death and Redwood National and State Parks¹

Monica Bueno,² Janelle Deshais,² and Leonel Arguello²

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
<u>Current efforts (<i>P. ramorum</i> not present)</u>		
Prevention	Education/Outreach BMPs for RNSP Information signs Public webpage Sanitation Gravel high-risk areas	To keep <i>P. ramorum</i> 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.
<u>Future potential efforts after <i>P. ramorum</i> arrives in park</u>		
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	To strengthen important trees near infection sites and to prevent human and natural transmission of the pathogen.
	AGRI-FOS®* – tanoaks	
	Physical treatments	
	Host removal in potential spread areas (buffers)	
Restoration/Conservation	Trail closures	To conserve ecologically important oaks and tanoaks in the landscape, rehabilitate infection sites, and minimize ecological impacts.
	Cleaning stations	
	Plant resistant hosts (if available)	
	Plant conifers and other species	
	Identify tanoak refuges and protect through physical and chemical buffers	

*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 Tissue¹

Gary Chastagner,² Annie DeBauw,² and Kathy Riley²

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 °C, 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.

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Effect of Surface Sterilization Treatments on the Detection and Viability of *Phytophthora ramorum* on Various Substrates¹

Katie Coats,² Kathy Riley,² Gary Chastagner,² and Marianne Elliott²

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 Crocker² and Matteo Garbelotto³

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 Entropy¹

Janelle Deshais,² John Stuart,² Steven Steinberg,³ and Leonel Arguello⁴

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 RNSP'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.

<i>Raster resolution</i>
10m ²
30m ²
<i>Species records*</i>
All available
Subset (excluded records without known GPS point)
<i>Environmental data</i>
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).

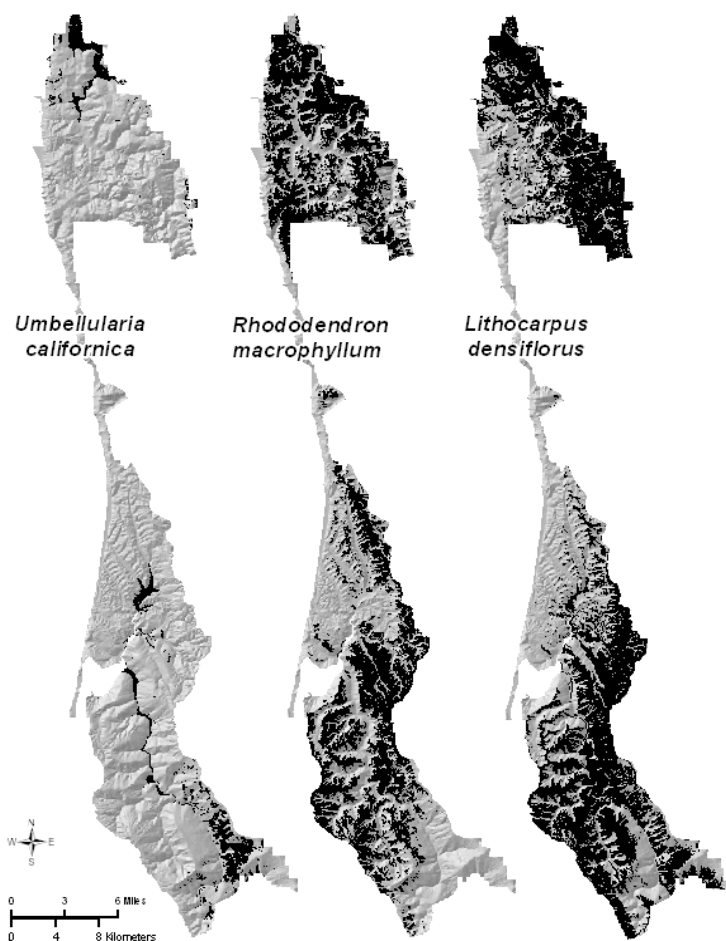


Figure 1—Presence of *Phytophthora ramorum* critical transmission host species in Redwood National and State Parks, California. Species' predicted presence is shown in black. Of the total 54,699 ha study site, our model predicted 3,361 ha (6 percent) with presence of *Umbellularia californica*, 21,804 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
<i>Umbellularia californica</i>	0.914 (0.019)	0.907 (0.020)	0.388*	<0.0001
<i>Rhododendron macrophyllum</i>	0.743 (0.017)	0.763 (0.083)	0.317**	<0.0001
<i>Lithocarpus densiflorus</i>	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 (%)
<i>Umbellularia californica</i> (10m ² grid resolution used)	Elevation	47.78
	Distance from ocean	20.54
	Soil type	14.31
	Degree slope	7.18
	Other	10.18
<i>Rhododendron macrophyllum</i> (10m ² grid resolution used)	Soil type	33.11
	Distance from ocean	21.48
	Elevation	12.08
	Slope position	11.03
	Other	22.30
<i>Lithocarpus densiflorus</i> (30m ² grid resolution used)	Soil type	35.32
	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 Lineages¹**

Clare R. Elliott,² Virginia McDonald,² and Niklaus J. Grünwald³

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 Hybrids¹

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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 mm².

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 sdH₂O and covered with nylon mesh to break surface tension. After 10 days, the disks were transferred to a 2 ml cryovial containing 1 ml sdH₂O 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.

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Phenotypic Variation in *Phytophthora ramorum*: Wild Type vs Non-Wild Type Isolates¹

Marianne Elliott,² Grace Sumampong,³ Simon F. Shamoun,³ Elisa Becker,³ Aniko Varga,⁴ Delano James,⁴ Saad Masri,⁴ and Niklaus J. Grünwald⁵

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.

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Ecology of *Phytophthora ramorum* in Watercourses: Implications for the Spread and Management of Sudden Oak Death¹

Elizabeth Fichtner,² Kamyar Aram,² and David Rizzo²

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 diplanetism 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 Forest¹

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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 10⁵ 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 Mountains¹

Kerri Frangioso,² Margaret Metz,² Allison Wickland,² David Rizzo,² and Ross Meentemeyer³

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 m² 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 Risk¹

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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 Ecoregion¹

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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 (*Quercus* 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 MicromaxTM incorporated at the rate of 1.75 lbs/yd³, 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 °C 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.

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Stream Baiting for Sudden Oak Death: Fluvial Transport and Ecohydrology of the Invasive Plant Pathogen *Phytophthora* *ramorum* in Western Washington State¹

Regina Johnson²

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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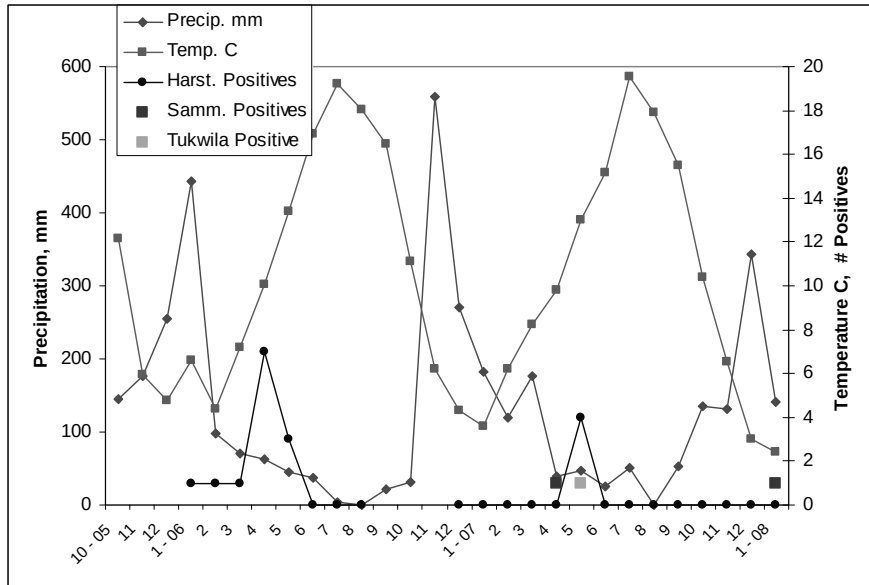


Figure 1—Precipitation, temperature, and positive stream baits for R Stream (Harstine), the Sammamish River, and Tukwila soil drainage ditch, 2006-07. Climate data for R Stream area from Western Regional Climate Center, <http://www.wrcc.dri.edu>. Climate data for Sammamish River/Tukwila ditch area are similar in pattern.

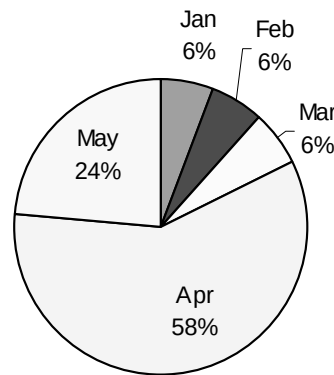


Figure 2— Positives in R Stream by month, 2006-2007.

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.

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Evaluation of Diurnal Rhythms in *Phytophthora*¹

Takao Kasuga² and Mai Bui²

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 Death¹

Maggi Kelly,² John Connors,² and Shufei Lei²

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 PostgreSQL. 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 Region¹

Sanjay Lamsal,² Ross K. Meentemeyer,² Qingmin Meng,² Margaret Metz,³ Richard Cobb,³ Kerri Frangioso,³ and David M. Rizzo³

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 CO₂.

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The California Oak Mortality Task Force and *Phytophthora ramorum* Outreach¹

Chris Lee,² Janice Alexander,³ and Katie Palmieri⁴

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* Diseases¹

Melissa Lujan,² Gary McAninch,² and Nancy Osterbauer²

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.

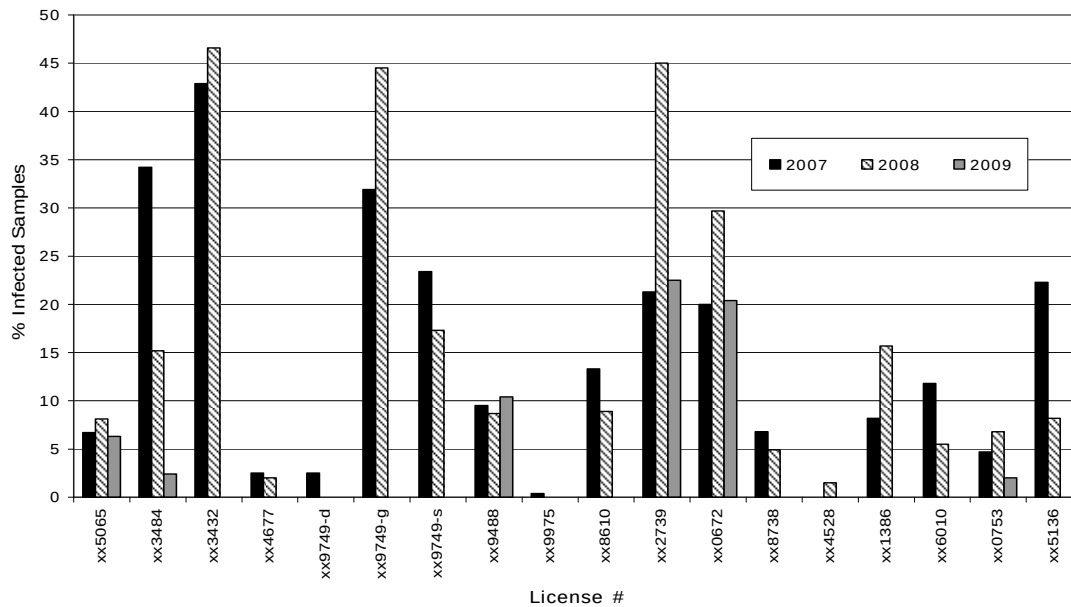


Figure 1—The percentage of aerial *Phytophthora* disease present at GAIP nurseries in 2007, 2008, and 2009.

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Effect of Plant Sterols and Tannins on *Phytophthora ramorum* Growth and Sporulation¹

Daniel Manter,² Eli Kolodny,² Rick Kelsey,³ and Pilar González-Hernández⁴

Abstract

The acquisition of plant sterols, mediated via elicitors, 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 elicitors, and a linear relationship was observed between the amount of elicitor 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 elicitors and sterol acquisition by *P. ramorum*.

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The Santa Lucia Preserve: A Case Study of Community-Based Sudden Oak Death Sampling Blitzes¹

Cheryl M. McCormick² and Matteo Garbelotto³

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 Oaks¹

Brice A. McPherson,² David L. Wood,² Nadir Erbilgin,³ and Pierluigi Bonello⁴

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, California¹

Brice A. McPherson,² David L. Wood,² Maggi Kelly,² and Gregory Biging²

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 Fire¹

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. Meentemeyer²

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 hyperspectral 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 Forests¹**

**Steven W. Oak,² Jaesoon Hwang,³ Steven N. Jeffers,³
and Borys M. Tkacz⁴**

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 Growers¹

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and Niklaus Grünwald⁶

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 Composition¹

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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 km² 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 Forests¹

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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 °C 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 (infested 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 <i>P.</i> <i>ramorum</i>	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 <i>P. ramorum</i>	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.

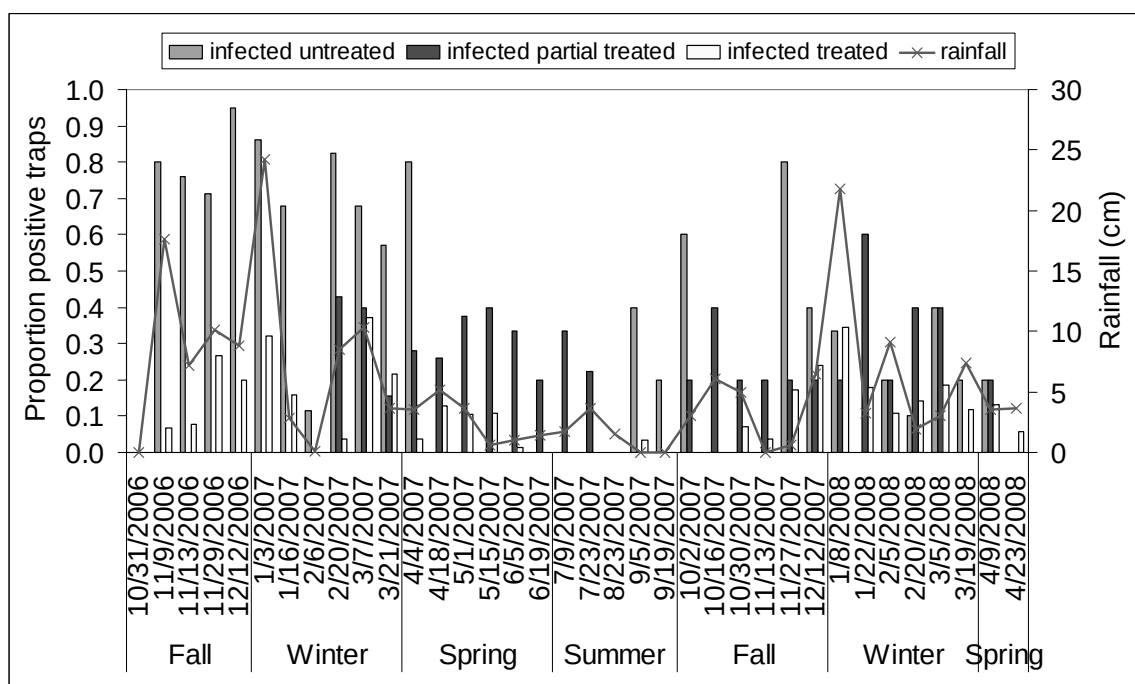


Figure 1—Inoculum was available during rain events throughout the year. *P. ramorum* was recovered from bait leaves in rain traps in 34 of 36 2-week sample periods. Periods with reduced rainfall (Red Mound Remote Automated Weather Station, <http://www.wrcc.dri.edu/cgi-bin/rawMAIN.pl?orOREM>) tended to 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.

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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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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).

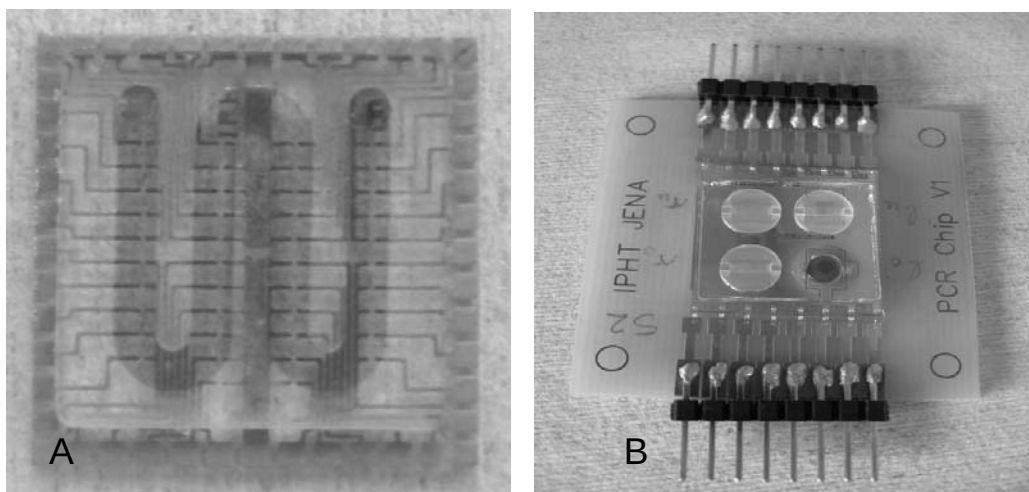


Figure 1—Stationary PCR chip with four integrated microstructured heaters, temperature sensors, and PDMS chamber for on-chip amplification and labeling of DNA (A); gold microarray chip combined with a microfluidic PDMS channel (prototype), specific oligonucleotide probes are spotted at 42 detection sites (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.

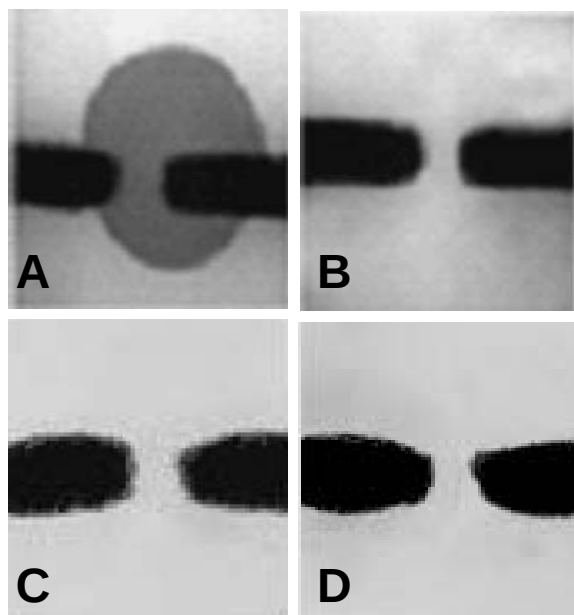


Figure 3—DNA chip: hybridization of *P. ramorum*-specific probe with ITS2 PCR products (ca 550bp) of *P. ramorum* and closely related *Phytophthora* taxa *P. lateralis* and *P. hibernalis* (optical analysis of gray scale value of the aggregated silver nanoparticles at electrode gap).

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 Flint⁴

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

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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 provisional 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 Forests¹

Wendy Sutton,² Everett Hansen,² Paul Reeser,² Alan Kanaskie,³
and Harvey Timeus³

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).

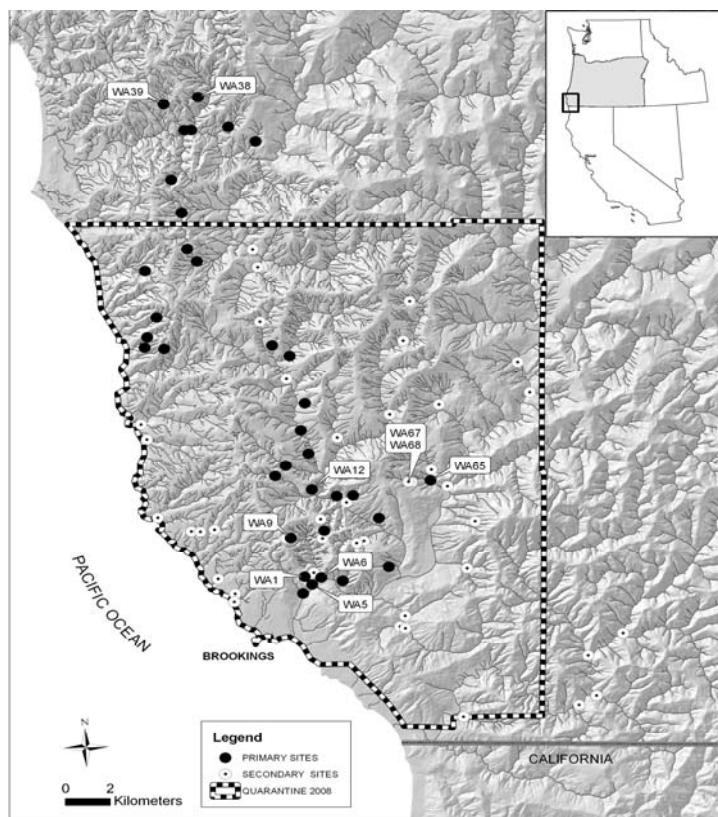


Figure 1—Map of southwest Oregon shows current quarantine area. Primary sites have been continuously sampled over time; secondary sites have been sampled varying lengths of time.

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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).

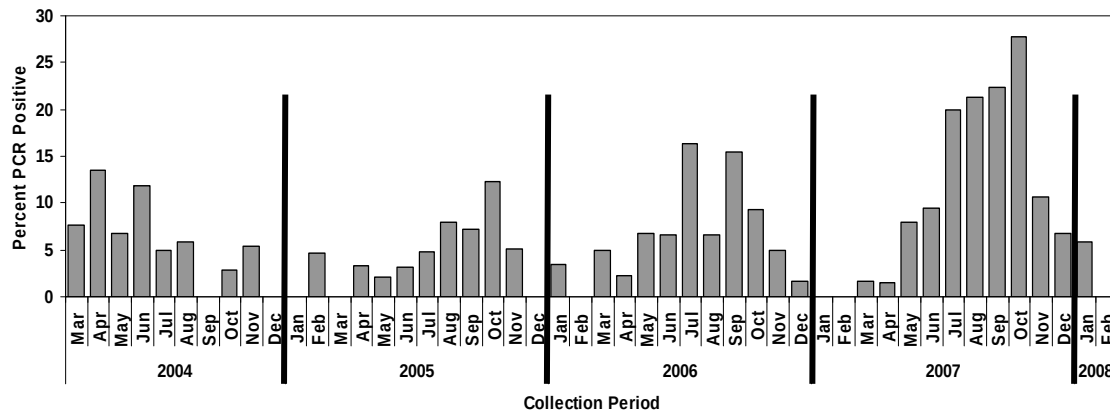


Figure 2—Detection of *P. ramorum* by multiplex PCR from leaf baits in 32 streams monitored continuously from March 2004 through February 2008. There were no samples collected in September 2004.

Periods of low recovery frequency corresponded with cooler water temperatures (fig. 3) and high winter flow rates in the streams (data not shown).

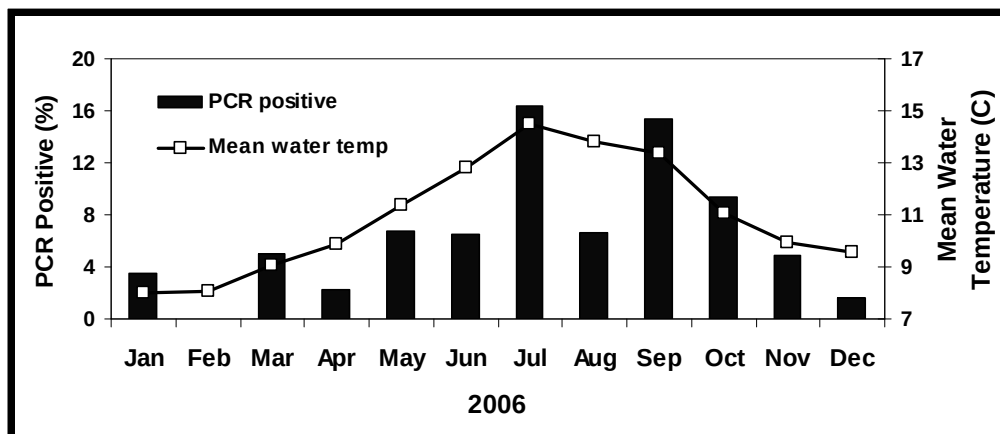


Figure 3—Monthly multiplex PCR detection of *P. ramorum* in leaf baits from 32 streams monitored in 2006 and water temperature of those streams.

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Factors Affecting Onset of Sporulation of *Phytophthora ramorum* on Rhododendron 'Cunningham's White'¹

Paul W. Tooley² and Marsha E. Browning²

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 dehiscent 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 mm²) corresponding to the initial collection of dehiscent 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, dehiscent 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. Widmer² and Gary J. Samuels³

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 °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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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*.

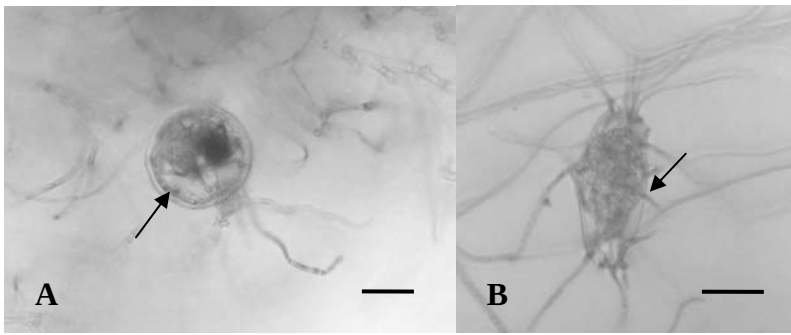


Figure 1—*Trichoderma asperellum* (arrows) demonstrating mycoparasitism of a *P. ramorum* **A**) chlamydospore and **B**) sporangium. Bar = 20 μ m.

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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