Phytophthora ramorum and Sudden Oak Death in California: I. Host Relationships[1]

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Abstract

A new canker disease of *Lithocarpus densiflorus*, *Quercus agrifolia*, *Q. kellogii*, and *Q. parvula* var. *shrevei* in California is shown to be caused by *Phytophthora ramorum*. The pathogen is a recently described species that was previously known only from Germany and The Netherlands on *Rhododendron* and *Viburnum*. This disease has reached epidemic proportions in mixed evergreen and redwood forests over an area approximately 300 km long along the central coast of California. The most consistent and diagnostic symptoms on larger trees are the cankers that develop before foliage symptoms become evident. Cankers have brown or black discolored bark, seep dark red sap and occur on the trunk at the root crown up to 20 m above the ground. Cankers do not enlarge below the soil line into the roots. Cankers can be over 2 m in length and are delimited by thin black zone lines in the inner bark. Foliage on affected trees often turns from a healthy green color to brown over a period of several weeks. In *L. densiflorus* saplings, *P. ramorum* was isolated from branches as small as 5 mm diameter. *Lithocarpus densiflorus* and *Q. agrifolia* inoculated with *P. ramorum* in the field and greenhouse developed symptoms similar to those of natural infections. The pathogen was re-isolated from inoculated plants, thereby confirming pathogenicity. Based on field observations and greenhouse inoculations, the host range of *P. ramorum* in California has been expanded and now includes *Rhododendron* spp., madrone (*Arbutus menziesii*), huckleberry (*Vaccinium ovatum*), manzanita (*Arctostaphylos* spp.), California bay laurel (*Umbellularia californica*), buckeye (*Aesculus californica*), bigleaf maple (*Acer macrophyllum*), toyon (*Heteromeles arbutifolia*), California coffeeberry (*Rhamnus californica*), and California honeysuckle (*Lonicera hispidula*). On these hosts, *P. ramorum* causes a variety of foliar and branch symptoms.

Introduction

Over the past 7 years, a disease of tanoak (*Lithocarpus densiflorus*), coast live oak (*Quercus agrifolia*) and California black oak (*Q. kelloggii*) has caused considerable mortality in central California (Garbelotto and others 2001, McPherson and others 2000). Named "Sudden Oak Death" in the popular press, the whole crown

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of affected trees often appears to die rapidly, and the foliage may turn from an apparently healthy green to brown within a few weeks. The most consistent and diagnostic symptom of the disease on larger trees is the development of cankers that have brown or black discolored bark on the lower trunk and seep dark red sap. The cankers do not extend into the roots. These cankers develop before foliage symptoms become evident. Such discoloration and bleeding are common symptoms associated with infection by *Phytophthora* species on oaks (Brasier and others 1993, Mircetich and others 1977, Tainter and others 2000). In agreement with this observation, we have consistently isolated the recently described *Phytophthora ramorum* from diseased *L. densiflorus* and *Quercus* spp. (Rizzo and others [In press]). The current range of the pathogen is considered to be from Big Sur in Monterey County to southern Oregon; the furthest inland sites are in western Solano County (Kelly 2002). Previously, *P. ramorum* had only been reported on ornamental rhododendron and viburnum in Germany and the Netherlands (Werres and others 2001). Field and greenhouse inoculations of tanoak and coast live oak seedlings, saplings, and mature trees produced symptoms similar to those observed with natural infections and confirmed the pathogenicity of *P. ramorum* (Rizzo and others [In press]).

To date, most work on this disease in California has concentrated on oaks. However, the finding of *P. ramorum* on rhododendron in Europe suggested that the host range of the pathogen in California needed further investigation. To understand the potential impact on oak trees we must have a thorough understanding of the biology of this pathogen including the complete host range, transmission dynamics and population biology of the pathogen. In this paper, we describe our efforts to determine the host range of *P. ramorum* in California including field isolations and inoculations of non-oak hosts. We also report on inoculations of two California white oak species from which the pathogen has not been found in the field, valley oak (*Q. lobata*) and blue oak (*Q. douglasii*). Two red oak species from eastern North American, northern red oak (*Quercus rubra*) and pin oak (*Quercus palustris*), were also included in these inoculations. In parts II (Davidson and others 2002) and III (Garbelotto and others 2002), we describe preliminary results on the transmission mechanisms and genetics of the pathogen, respectively. Finally, in part IV (Garbelotto and others 2002), we discuss initial results from chemical control studies of *P. ramorum*.

Methods

Field Survey

Symptomatic and dead trees and shrubs were examined throughout the known range of *P. ramorum*. At each site, samples were taken from trees that matched symptoms of *Phytophthora* infection and returned to the laboratory for isolation. Specific locations of sampled hosts were marked using global positioning equipment (GPS, Trimble Corp., Sunnyvale, California) and entered into a monitoring database maintained by Dr. Maggi Kelly, University of California, Berkeley (http://camfer.cnr.berkeley.edu/oaks/). Additional data collected with each sample included host species, host symptoms, tree diameter, forest type, overall stand health, and relationship to other symptomatic trees. Where possible, samples were collected from multiple hosts at each location.

Isolation methods for *P. ramorum* on oaks are described elsewhere (Rizzo and others [In press]). For hosts with foliar lesions, discolored and necrotic areas were

excised from leaves and placed in petri dishes (either in the laboratory or in the field) containing pimaricin-ampicillin-rifampicin-PCNB agar (PARP), a selective medium for *Phytophthora* species (Erwin and Ribeiro 1996). Plates were incubated in the dark at 20 to 22°C and examined within 2 to 5 days. We have also developed PCR (polymerase chain reaction) primers based on the nucleotide sequences of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA that are specific for *P. ramorum* (Garbelotto and others 2002). We have used these primers to successfully amplify DNA from infected leaves of multiple hosts and have used this methodology to pre-screen most foliar hosts (Garbelotto and others 2002).

Inoculation Trials

Greenhouse trials were conducted on seedlings of potential host species to evaluate the pathogenicity of *P. ramorum*. Inoculum was prepared by growing isolates on V-8 juice agar for 7 days, then cutting out 5 mm diameter plugs using a sterilized cork borer. Three isolates of *P. ramorum* were used for all tests: Pr-5 from tanoak, Pr-6 from coast live oak, and Pr-52 from rhododendron. Methods for stem inoculations of oak seedlings are described elsewhere (Rizzo and others [In press]). Foliar hosts were selected based on results of positive field isolations; a complete range of all woody plants found in oak forests have not been tested. Plants were purchased from native plant nurseries and were typically 2-3 years old. Foliage inoculations were conducted by misting leaves with sterile distilled water (SDW) and then pinning inoculum plugs to the upper surface of leaves. Sterile agar plugs were used as controls. A zip-loc plastic bag was then placed over the individual leaves; prior to sealing the bag the leaves were misted again with SDW. Each trial consisted of 10 leaves per host species per isolate plus controls. For most trials, the three isolates of *P. ramorum* listed above were used. On each of five seedlings, two leaves per isolate plus two control leaves were inoculated. Seedlings were incubated for 2 weeks in a greenhouse that was maintained at 22 to 24°C. For each trial, symptoms were recorded, lesion length and width was measured and pieces of stems or leaves were plated on PARP to verify presence or absence of *P. ramorum*. All leaf inoculations were conducted twice.

Results

Field Isolations

We have recovered *P. ramorum* via isolation and direct PCR from symptomatic plant tissue of the following tree species: madrone (*Arbutus menziesii*), California bay laurel (*Umbellularia californica*), buckeye (*Aesculus californica*), huckleberry (*Vaccinium ovatum*) and *Rhododendron* spp. (cultivars "Gomer Waterer" and "Colonel Coen," *R. macrophyllum*) *(table 1)*. Using the direct PCR method on symptomatic leaf tissue, we have also detected *P. ramorum* DNA from field samples of manzanita (*Arctostaphylos manzanita*), bigleaf maple (*Acer macrophyllum*), toyon (*Heteromeles arbutifolia*), California coffeeberry (*Rhamnus californica*), and California honeysuckle (*Lonicera hispidula*). However, at the present time, we have been unable to isolate *P. ramorum* from these latter hosts. In all cases, *P. ramorum* was recovered from aboveground plant parts such as leaves or branches *(table 1)*. Of the non-oak hosts, madrone, manzanita, rhododendron, and huckleberry appear to show the most dieback associated with the disease in the field.

Table 1—Known hosts of *Phytophthora ramorum* in California and their method of detection in the field.

			Method of detection[2]	
Host[1]	**Common name**	**Plant part infected**	**Field isolation**	**Direct PCR**
Quercus agrifolia (Fagaceae)	Coast live oak	main stem	+	+
Q. kellogii (Fagaceae)	California black oak	main stem	+	+
Q. parvula var. *shrevei* (Fagaceae)	Shreve's oak	main stem	+	nt
Lithocarpus densiflorus (Fagaceae)	Tanoak	main stem, branches, leaves	+	+
Umbellaria californica (Lauraceae)	Bay laurel	leaves	+	+
Arbutus menziesii (Ericaceae)	Madrone	branches, leaves	+	+
Vaccinium ovatum (Ericaceae)	Huckleberry	main stem, branches, leaves	+	+
Arctostaphylos spp. (Ericaceae)	Manzanita	branches, leaves	-	+
Rhododendron spp. (Ericaceae)	Rhododendron	branches, leaves	+	+
Aesculus californica (Hippocastanaceae)	Buckeye	branches, leaves	+	+
Acer macrophyllum (Aceraceae)	Big leaf maple	leaves	-	+
Heteromeles arbutifolia (Rosaceae)	Toyon	branches, leaves	-	+
Rhamnus californica (Rhamnaceae)	California coffeeberry	leaves	-	+
Lonicera hispidula (Caprifoliaceae)	California honeysuckle	leaves	-	+

[1] *Rhododendron* spp. and *Viburnum* sp. have been found as hosts of *P. ramorum* in The Netherlands and Germany.

[2] Symbols: +, positive isolation or detection by PCR; -, tested but not recovered with this method to date; nt, not tested.

Seedling Inoculation Trials

In all hosts tested, *P. ramorum* was reisolated after two weeks from inoculated leaves and not from control leaves *(table 2)*. With the two *Quercus* species, lesions were no larger than a typical control wound after 2 weeks (mean width, 1.9 mm), however *P. ramorum* was still reisolated. Mean lesion width on bay and toyon was only slightly larger than found on oak (mean lesion width, 5 mm). Lesions on huckleberry, manzanita, and tanoak often covered the entire inoculated leaf and in some cases, the pathogen grew through the petioles to colonize branches and stems. With madrone and bigleaf maple, lesions were often over 4 cm in diameter.

Table 2—*Pathogenicity of* Phytophthora ramorum *on leaves of selected native California plant species.*

	Disease rating[1]	
Host	***P. ramorum***	**Control**
Quercus agrifolia	+	-
Q. kellogii	+	-
Lithocarpus densiflorus	+++	-
Umbellaria californica	++	-
Arbutus menziesii	+++	-
Vaccinium ovatum	+++	-
Arctostaphylos manzanita	+++	-
Rhododendron macrophyllum	+++	-
Aesculus californica	++	-
Acer macrophyllum	+++	-
Heteromeles arbutifolia	++	-

[1] Plants were evaluated after 2 weeks using the following disease rating scale: -, < 5 mm lesion around pin, *P. ramorum* not isolated; +, < 5 mm lesion around pin, *P. ramorum* isolated; ++, 5 mm to 20 mm leaf spots, *P. ramorum* isolated; +++, > 20 mm leafspots; often entire leaf colonized or discolored, *P. ramorum* isolated.

In the oak seedling trial, *P. ramorum* caused lesions that were significantly greater than found on the controls in each of the red oak species and tanoak, but not in the two white oak species (ANOVA, $P < 0.05$) *(table 3)*. In the seedlings inoculated with *P. ramorum*, the external surface of the bark was sunken along the entire length of colonization by the pathogen. Internal discoloration was generally limited to the phloem although some xylem discoloration was apparent. *Phytophthora* was recovered from all inoculated stems, including valley oak and blue oak, and none of the controls. The results of the inoculations of tanoak and coast live oak in this study are consistent with those reported in a previous experiment (Rizzo and others [In press]).

Table 3—*Mean and range in lesion length in tanoak, red oak and white oak seedlings resulting from inoculations with Phytophthora ramorum.*

	Mean length of discoloration, cm[1] (range of discoloration)	
Host	***P. ramorum* N=30**	**Control N=10**
Lithocarpus densiflorus	4.7 (1.7 – 21.0)	1.5 (0.9 – 4.0)
Quercus agrifolia (red oak group)	2.3 (1.3 – 4.8)	1.0 (0.7 – 1.4)
Quercus kellogii (red oak group)	3.1 (1.5 – 8.0)	1.3 (1.1 – 1.5)
Quercus rubra (red oak group)	4.4 (1.2 – 9.7)	1.4 (1.0 –2.0)
Quercus palustris (red oak group)	3.3 (0.7 – 8.1)	1.3 (1.1 – 1.5)
Quercus douglasii (white oak group)	1.3 (0.7 – 2.4)	0.8 (0.3 – 1.3)
Quercus lobata (white oak group)	1.4 (0.8 – 2.2)	1.0 (0.6 – 1.6)

[1] Thirty seedlings of each host species were inoculated with three isolates of *P. ramorum* (10 seedlings per isolate). Data for all three isolates are combined in the table. *Phytophthora ramorum* was recovered from all inoculations including *Q. douglasii* and *Q. lobata*; *P. ramorum* was not recovered from the control inoculations. Based on ANOVA, lesion lengths were significantly different between *P. ramorum*-inoculated seedlings and controls except for *Q. douglasii* and *Q. lobata*.

Discussion

These data clearly indicate that *P. ramorum* infects many different plant species in addition to oaks and tanoak in coastal California forests. In some mixed-evergreen forests nearly all woody plants can serve as host for *P. ramorum*. For example, at China Camp State Park in Marin County, the overstory consists of coast live oak, black oak, bay laurel, and madrone. Toyon is the primary understory shrub species. Buckeye and manzanita are found at the margins of closed canopy. Valley oak (in riparian areas and at the edge of the closed canopy) appears to be the only woody plant species unaffected by *P. ramorum* at this location. There are many other plant species that co-occur with oaks and tanoak that have not been tested for susceptibility to *P. ramorum*. This includes additional species in the Ericaceae (e.g., salal). Species in this family appear to be the most affected after the oaks. As the geographic range of *P. ramorum* changes, it will potentially encounter other hosts that are not found in forests in the current range.

Exotic species of *Phytophthora* have been considered responsible for extensive tree mortality and negative ecological impact in forests of Australia, Europe and

North America (Brasier 2000, Hansen 2000, Old and Dudzinski 2000). *Phytophthora cinnamomi* has devastated the jarrah forests of western Australia, killing over 50 percent of all plant species over several hundred thousand hectares (Weste and Marks 1983). The broad host range of *P. ramorum* that we have discovered in California forests suggest that this pathogen has the potential to cause similar, long-term landscape level changes in these forests. Loss of oaks and other overstory trees and shrubs will have cascading effects on these ecosystems including increased fire hazards, soil erosion, and loss of habitat for wildlife (McPherson and others 2000, Garbelotto and others 2001). Developing restoration plans for these ecosystems will represent a significant future challenge for forest managers.

The most encouraging result from our inoculation study was the finding that *P. ramorum* did not cause lesions on valley oak and blue oak that differed significantly from the control wounds. However, the pathogen did survive in the discolored tissue for at least 6 weeks following inoculation. It is unknown if the pathogen can continue to survive for long periods in white oak tissue and eventually cause mortality of plants. To date, no species in the white oak group has been diagnosed with the disease in the field. Further studies in the greenhouse are planned.

We report our initial results of a study comparing the susceptibility of California oaks and eastern North American oak species to *P. ramorum*. Challenging seedlings of northern red oak (*Quercus rubra*) and pin oak (*Quercus palustris*) with *P. ramorum* resulted in longer lesions in the bark than those that developed in the coast live oak and California black oak. Lesions in northern red oak were nearly twice as long as those observed in coast live oak and about the same length as those observed in tanoak. Tanoak is considered to be the most susceptible California tree species to *P. ramorum*. Extrapolation of results from seedling experiments to the potential effects on mature trees must be done cautiously. However, because lesion sizes in red oak and pin oak seedlings were much larger than in coast live oak seedlings (a species in which the adults are very susceptible), we suggest that it is likely that mature trees of northern red oak and pin oak will be susceptible to infection by *P. ramorum*. Therefore, efforts to prevent spread of *P. ramorum* to eastern North American forests are critical.

An understanding of the host range and spatial distribution of the pathogen is pivotal in formulating hypotheses for further epidemiological research (e.g. Davidson and others 2002), developing monitoring strategies and management guidelines that may either prevent further spread of the disease or ameliorate disease conditions where the disease may be only recently present.

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