Report on coast redwood and Douglas-fir as hosts for *Phytophthora ramorum*

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Redwood

Phytophthora ramorum has been isolated from discolored needles and cankers on small branches (<0.5 cm diam) on 27 coast redwood (*Sequoia sempervirens*) saplings (2 to 17 cm diameter) at two locations in California (Jack London State Park, Sonoma Co. and Henry Cowell State Park, Santa Cruz Co.). Symptoms were observed on branches throughout the crowns of the affected trees. Isolates were identified as *P. ramorum* by their abundant chlamydospores and caducous, semi-papillate sporangia and identical ITS rDNA sequences to isolates of *P. ramorum* from *Quercus* spp., *Lithocarpus densiflora,* and *Rhododendron*. *P. ramorum* was also detected in dying basal sprouts on mature redwood trees from an additional five locations in coastal California by PCR amplification of the ITS region using DNA extracted from symptomatic tissue and *P. ramorum*-specific PCR primers.

Table 1. Locations where *Phytophthora ramorum* has been detected in California.

Location	County	Identification method
Muir Woods	Marin	PCR
Lake Lagunitas, Marin Municipal Water District	Marin	PCR
UC Berkeley campus	Alameda	PCR
Jack London State Park	Sonoma	PCR, isolation
Armstrong Redwoods State Park	Sonoma	PCR
Henry Cowell Sate Park	Santa Cruz	PCR, isolation
Pfeiffer/Big Sur State Park	Monterey	PCR

To test for pathogenicity, foliage inoculations were conducted on redwood seedlings in two trials by misting 30 needles per trial (5 needles per seedling plus controls) with sterile distilled water and then pinning inoculum plugs to the upper surface of needles. Inoculation resulted in lesions of 1 to 20 mm on individual needles and *P. ramorum* was recovered from 43 percent of the inoculated needles. Symptoms were not restricted to the inoculated leaves; 15 inoculations of individual leaves led to discoloration of 2 or more adjacent leaves. On one inoculation, 60 mm of the adjacent stem was killed. Redwood seedling stems (diameter approximately 1 cm) were wound inoculated in two trials (10 seedlings per trial plus controls). After 6 weeks, lesion lengths in the cambium averaged 13.7 mm (range 4 to 21 mm). *P. ramorum* was recovered from 100 % of the inoculations. Entire branches near the inoculation

became chlorotic even though no direct connection was seen between the lesion and the branches; such chlorosis was not observed in control inoculations. Mean lesion lengths of *P. ramorum* were significantly greater in both trials than those of control inoculations at $P < 0.05$ based on ANOVA. Redwood saplings (2.5 to 4.5 cm diam) were also wound inoculated in a separate trial. After 7 weeks, no phloem or cambial discoloration was noted, but necrotic lesions in the xylem had a mean length of 39 mm (range12 to 73 mm). In addition, narrow streaks, 1-2 mm diameter, were also noted in the xylem extending from the necrotic areas upward to 90 cm. Recovery of *P. ramorum* from inoculations was 70 %. Mean lesion lengths of *P. ramorum* were significantly greater in all trials than those of control inoculations at $P < 0.05$ based on ANOVA.

In January 2002, dieback of a mature redwood in an urban setting in Mill Valley was reported in the popular press and attributed to infection by *P. ramorum*. This diagnosis was conducted by a private laboratory and based on detection of a *Phytophthora* species using a non-specific immuno-assay. We have confirmed that *P. ramorum* was present in the discolored xylem of the stump of the tree using direct PCR amplification. We were unable to directly isolate the pathogen from the discolored tissue, nor bait the pathogen from bark or soil samples taken from the base of the tree. This tree was impacted by a concrete patio and driveway and had extensive root rot by two unidentified wood-decay fungi and *Armillaria mellea*. The role of *P. ramorum* in the development of dieback symptoms on this tree is difficult to determine because of the presence of these other pathogens and abiotic factors. Three other urban redwood trees in Ross also reported in the popular press to be associated with *P. ramorum* were negative for the pathogen both by PCR analysis and culturing, although all of these trees had extensive decay caused by *Armillaria mellea* and an unidentified canker rot fungus.

The importance of *P. ramorum* in dieback and death of mature redwood is uncertain. We have not observed unusual mortality or disease symptoms on overstory redwoods in natural forests. The observation of *P. ramorum* in the xylem of a large *S. sempevirens* and the streaking observed in the stem inoculations of redwood saplings suggests that the situation bears more research, but it would be inappropriate to draw general conclusions about the ability of *P. ramorum* to infect larger redwood trees in the field. The impact of infection by *P. ramorum* on understory redwoods is also unclear at this time. Infection appears to be common on needles and very small branches of understory redwood at many sites and *P. ramorum* appears to be able to kill sprouts and may cause a loss of vigor in saplings. However, the long term implications for redwood regeneration are unknown.

Douglas-fir

We have recovered *P. ramorum* from infected branch tips of Douglas-fir (*Pseudotsuga menziesii*) saplings at the Fairfield Osborn Preserve in Sonoma County. Symptoms on these saplings included cankers on small branches (0.5 to 1 cm diameter) resulting in wilting of new shoots, dieback of branches and loss of needles up to 15 cm from the twig tip. Symptoms were observed on most saplings growing in the same area. On several smaller saplings (<1 m tall), *P. ramorum* infection resulted in the death of the leader and the top several whorls of branches. Isolates were identified as *P. ramorum* by their abundant chlamydospores and caducous, semi-papillate sporangia and identical ITS sequences to isolates of *P. ramorum* from *Quercus* spp., *Lithocarpus densiflora,* and *Rhododendron*.

To test for pathogenicity, foliage inoculations were conducted as described above for coast redwood. Inoculation resulted in lesions ranging between 1 and 12 mm in length, and *P.*

ramorum was recovered from 47 percent of the inoculated needles. Symptoms were not restricted to the inoculated needles, and in 26 single needle inoculations, lesions 17-85 mm long developed on branches (5 mm in diameter) adjacent to the inoculated needle. Isolation success from branch lesions was 50%, in spite of the fact such lesions were apparently disjunct from the small 1-mm lesions developing on the inoculated needles. Stems of Douglas-fir seedlings (diameter approximately 1 cm) were wound inoculated in two separate trials (10 seedlings per trial plus controls). After 6 weeks, lesions lengths in the cambium averaged 38 mm (range 12 to 62 mm) and three seedlings were completely girdled. *P. ramorum* was recovered from 75% of the inoculations. Mean lesion lengths of *P. ramorum* were significantly greater in both trials than those of control inoculations at $P < 0.05$ based on ANOVA.

We have not observed unusual mortality or disease symptoms on overstory Douglas fir trees in natural forests. The importance of *P. ramorum* branch tip dieback for growth and reproduction of Douglas-fir is unknown. Douglas-fir is present in many forests in California and Oregon already infested by *P. ramorum*, yet we have found infection of plants at only one location. At this site, the symptomatic Douglas-fir saplings were surrounded by bay laurel (*Umbellularia californica*) trees with extremely high levels of *P. ramorum* infection. *P. ramorum* is known to sporulate prolifically on bay laurel leaves. More studies are necessary to determine if the incidence of *P. ramorum* in Douglas-fir extends to other locations or if it is limited to this one locale.

Summary

For both redwood and Douglas-fir, the ultimate ecological impact of *P. ramorum* infection is unknown. We have no evidence at this time that large overstory trees can be infected by the pathogen. All isolations of the pathogen in the field have been from seedlings or saplings. The direct impact of *P. ramorum* infection on individual plants appears greater for Douglas-fir than redwood. However, the long-term implications for the health of individual plants is unknown for both host species. The role these hosts play in the epidemiology (e.g., ability to support sporulation) of *P. ramorum* is also unknown.

We also do not know the risks associated with these two hosts for disease spread through human activity. To date, only small plants or small plant parts (less than 4 inches diameter) have been shown to be infected for both tree species. As mentioned above, we have detected the pathogen in a large tree in only one instance. In this case, the redwood tree was severely compromised by cutting and the presence of other pathogens, making it impossible to evaluate the role of *P. ramorum* in this situation.

Redwood and Douglas-fir are clearly hosts for *P. ramorum*, but we have only studied the biology of *P. ramorum* on these hosts for several months. Much more work is needed to answer most questions concerning ecological impacts on the two new hosts and their potential role in the epidemiology of the “sudden oak death’ disease.

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Symptoms on Redwood

Lesions on individual needles of redwood sapling. Note new foliage does not appear affected.

Typical *P. ramorum* lesion on redwood sprout. Other pathogens may also cause similar lesions.

Symptoms on Douglas-fir.

Wilting of branch tips with new foliage on Douglas-fir.

Wilting of branch tips and death of main leader and side branches.