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

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Abstract

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Literature Cited

Denman, S.; Kirk, S.A.; Brasier, C.M.; Webber, J.F. 2005. *In vitro* leaf inoculation studies as an indication of tree foliage susceptibility to *Phytophthora ramorum* in the U.K. Plant Pathology. 54: 512–521.

Hansen, E.M.; Parke, J.L.; Sutton, W. 2005. Susceptibility of Oregon forests trees and shrubs to *Phytophthora ramorum*: a comparison of artificial inoculation and natural infections. Plant Disease. 89(1): 63–70.