New Insights into the Ecology of *Phytophthora ramorum* in Streams[1]

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Abstract

Many *Phytophthora* species, including *Phytophthora ramorum,* have been reported from surface waters such as canals, streams, rivers, ponds, and reservoirs, often in association with infested agricultural or natural landscapes (Hong and Moorman 2005). *Phytophthora* species are recovered with regularity and abundance from streams and rivers, in many cases in the absence of apparent terrestrial infestations (Hwang et al. 2010, Reeser et al. 2011, Sutton et al. 2009), or under conditions not conducive to sporulation in the landscape in the case of California summers. This strongly suggests that these organisms can complete their life cycle in aquatic environments, yet the biological underpinnings of this phenomenon remain effectively unknown. What *Phytophthora* propagules occur in surface waters? How frequent and widespread are they in such environments? From what substrates do they originate? What is their potential to infect plant material in this environment? To address these questions, we have undertaken experiments focusing on *P. ramorum* in both naturally infested California streams and controlled environments. The persistence of such plant pathogens in water bodies has significant implications for their spread and management in both agricultural and more natural contexts.

In the absence of terrestrial run-off, *Phytophthora* inoculum in waterways may originate from infections of leaf litter or aquatic plants. Though fresh leaf litter can serve as a transient substrate for growth and reproduction of *Phytophthora* spp. in streams, most vegetative litter quickly degrades in an aquatic environment. To determine the potential of leaf litter as a substrate for *P. ramorum* in streams, we exposed freshly picked rhododendron leaves, as well as those killed by drying or freezing, to natural inoculum in infested streams and additionally to laboratory-produced inoculum in controlled environment experiments. Baits were deployed monthly during peak pathogen activity from January to June in each of 2 years. *Phytophthora ramorum* was recovered by culturing from 62 percent of fresh leaves, but only 6 percent and 2 percent of frozen and dried leaves, respectively (n=298 per treatment). In laboratory tests, we incubated fresh, frozen, and dried leaves separately in cups of water inoculated with *P. ramorum* sporangia or zoospores for 7 days at 16 ^{o}C. Measuring the proportion colonized by sampling the surface of each leaf in a mosaic pattern, we found that *P. ramorum* colonized an average of 82 percent of fresh rhododendron leaf surfaces, but no more than an average of 30 percent for frozen and dried leaves (n=12 per treatment). Furthermore, when *P. ramorum* was incubated in similar laboratory experiments with spores of *Phytophthora gonapodyides*, a primarily saprobic, aquatic species, *P. ramorum* colonized an average of 68 percent, 10 percent, and 3 percent, while *P. gonapodyides* was recovered from an average of 0 percent, 78 percent, and 58 percent of fresh, frozen and dried leaves, respectively (n=12 per treatment), suggesting competition between the two species. These results indicated that *P. ramorum* may be limited biologically and ecologically from colonizing degraded leaf litter in aquatic environments.

We also examined colonization of naturally occurring leaf litter in an infested stream through mosaic sampling of individual leaves. *Phytophthora ramorum* was primarily recovered from bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.), and never from coast live oak (*Quercus agrifolia* Née) leaf litter. In one instance, it was recovered from alder *(Alnus* sp.) leaves from the stream. A portion of bay laurel litter leaves were sorted either as fresh (some green tissue, more supple) or degraded (mostly brown and thin). Both litter types were colonized by *P. ramorum* at similar levels. It is therefore possible that *P. ramorum* may persist on infected leaves as they degrade, even if it is not effective at colonizing already degraded leaves.

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To determine if *P. ramorum* propagules can adhere passively to substrates in streams, we incubated glass microscope slides in two naturally infested streams. Colonies of *P. ramorum* and *P. gonapodyides* were recovered from glass slides exposed in streams by submerging them into cooling selective media before it solidified, indicating that passive adherence of propagules to substrates in streams occurs.

Aquatic and riparian plants were evaluated as a potential source of *P. ramorum* inoculum. More than 100 specimens of aquatic plants representing more than 20 families were collected from within and near the edge of streams where *P. ramorum* had regularly been detected in the water, but not on the landscape. They included entire plants, submerged branches (sometimes rooting in the stream), or roots only. Both herbaceous and woody plants were sampled. Specimens were surface sterilized in 5 percent household bleach for 2 minutes, then kept in a few cm of water in partially sealed plastic bags, covered with a large plastic bag to keep in moisture, and incubated in a growth chamber at 16/12 ^{o}C, corresponding to 14/10 hours light/dark. We maintained plant material for 3 to 5 days with all but a little water drained from the containing bags to produce conditions conducive to sporulation prior to flooding and baiting them with rhododendron leaves. *P. gonapodyides* and other unidentified Pythiaceous species were baited from some plants, but *P. ramorum* was not recovered from any of the specimens.

Nonetheless, we found by direct isolation that leaves of chain fern (*Woodwardia fimbriata* Sm.) naturally submerged in an infested stream were infected with *P. ramorum* and *P. gonapodyides*. However, recovery from this previously unreported host was irregular and it is uncertain if such infections contribute significant inoculum to the stream.

These results suggest a complex ecological picture in regard to leaf litter and aquatic plants as potential substrates for the persistence and reproduction of *P. ramorum* in streams. While *P. ramorum* seems incapable of significant colonization of degraded leaf litter, it may persist on previously colonized leaves as they degrade further. The isolation of *P. ramorum* from a non-living substrate such as glass shows that spores are abundant and passively encounter and adhere to substrates. Further study is needed to determine the importance of infected leaf litter in dispersing and supporting spore loads downstream of infested terrestrial areas. It remains to be determined whether infections of typically non-host species, such as alder, can play a role in sustaining an active presence of *P. ramorum* in aquatic environments. None of the aquatic plants that were kept in growth chambers were infected by *P. ramorum* at a level detectable by baiting, though the detection of other *Phytophthora* spp. suggests that the method was effective. Further work is underway to test the specimens with direct inoculation. The isolation of *P. ramorum* and *P. gonapodyides* from chain fern shows that aquatic plants, like leaf litter or bait, can become infected in streams, but their significance as an inoculum source may be limited or transient. Nonetheless, the presence of such transitory substrates such as leaf litter and submerged leaves of aquatic plants may play a role in the seasonal fluctuation of spore loads observed in California streams. Bay laurel leaves are often shed in abundance in late spring and rising water levels often submerge drooping leaves of riparian vegetation. Such substrates may become mostly exhausted later in the summer by which time detection of *P. ramorum* in streams also declines.

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