

Monitoring *Phytophthora ramorum* Distribution in Streams within California Watersheds

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ABSTRACT

One hundred eighty-one sites were established in perennial watercourses and sampled for one to four years between 2004 and 2007 to monitor for the presence of *Phytophthora ramorum* (Pr) throughout coastal central and northern California watersheds as well as portions of the Sierra Nevada mountain range. In 2007, 132 sites were monitored, including 65 new sites. The majority of the monitored watersheds have limited or no Pr at this time, but are near the epidemic range of Pr and/or are considered high-risk for invasion by Pr. Three currently infested watersheds in Sonoma, Humboldt, and Monterey Counties were included as a baseline for successful recovery of Pr. *Rhododendron* leaves were placed in mesh bags and secured in watercourses for 1 to 3 week intervals to bait for *Phytophthora* species (Fig. 1). Recovered symptomatic leaves were plated on *Phytophthora*-selective medium (PARP-H) and monitored microscopically (Fig. 2).

Pr has been detected at 37 total stream monitoring sites, including all sites with *a priori* knowledge of nearby forest infestation (Fig. 3). Pr was detected at 23 streams sites without prior knowledge of adjacent forest infestation in Humboldt, Contra Costa, Mendocino, Monterey, and Santa Cruz counties. Forest infestations have thus far been confirmed at only nine of these sites; surveys are underway to identify the source(s) of inoculum for the other sites. Additionally, Pr was recovered as far as 25km downstream from known forest infestations. This year (2007) was an unusually dry year in California which impacted our recovery of Pr from watercourses. Pr was detected in only 15 streams this year; seven of those were new sites for 2007. We recovered no Pr from four streams that were positive for Pr in 2006. At the most heavily infested sites, the frequency and quantity of recovery of Pr was greatly reduced in 2007.

At two sites in 2006 we observed Pr infected plant lesions at high water levels, indicating the possibility of pathogen transmission back onto land from infected water courses. Stream monitoring has extended the southern range of Pr in Monterey County and the northern range in Humboldt County. All sites in the Sierra Nevada remain negative for Pr. With culturing and molecular sequencing we have identified several other *Phytophthora* species within these watersheds; *P. gonapodyides* is the most commonly detected species and was isolated from over 60 sites. Streams were monitored year-round in 2004 and 2005 and revealed a distinct seasonality associated with Pr recovery (Fig. 4). Therefore, in 2006 and 2007 watersheds were monitored monthly, February through June, during the peak seasonal period.

Stream monitoring provides a useful method of early detection for Pr infestation in watersheds. A portion of this work is part of the national Pr stream monitoring program supported by the US Forest Service. This project involves many collaborators who's assistance, permission, and guidance make this work possible.

METHODS

- Clean, disease-free *Rhododendron* leaves are placed in 1mm sterilized fiberglass mesh bags at stream sites once per month February-June
- Ten leaves are placed at each location with two replicate locations per site.
- Bags are secured to streambanks and floated near the water surface (von Broembsen 2002) for 7-21 days with the minimum time period in warm stream and air temperatures and longer intervals in cold conditions. Interval time is adjusted year-round.
- Leaves are collected and placed in separate sample collection bags.
- Leaves are surface sterilized in 95% Ethanol for 30 seconds, rinsed with DI water, and air-dried for 1 hour.
- Disease symptoms are described and recorded for all leaves.
- Symptomatic leaves are isolated onto PARP with 0.025g/L hymexazol, known to reduce *Pythium* species growth without impacting *Phytophthora* growth. Experiments have shown minimal inhibition of *P. ramorum* growth with this concentration of hymexazol (Fichtner *et al* 2005). Experiments were conducted to examine hymexazol inhibition on other *Phytophthora* species (unpublished data).
- Plates are incubated at 18°C for three weeks and checked microscopically twice weekly for growth of *Phytophthora* species; any *Phytophthora*-like organisms are transferred and further examined for identification



Figure 1. Methods demonstration

RESULTS

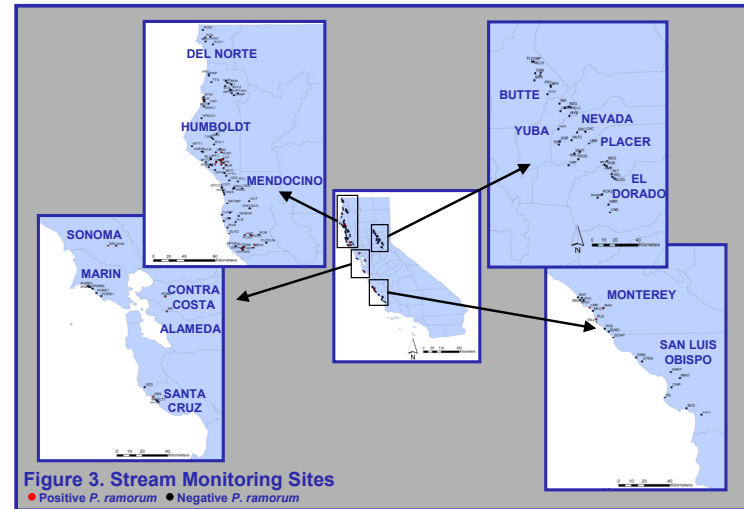


Figure 3. Stream Monitoring Sites

● Positive *P. ramorum* ● Negative *P. ramorum*

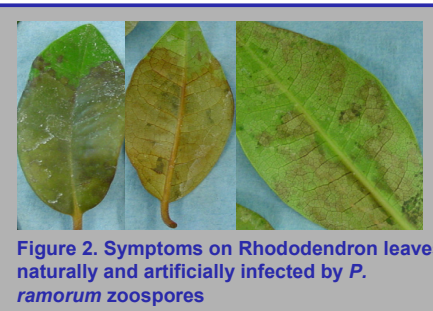


Figure 2. Symptoms on *Rhododendron* leaves naturally and artificially infected by *P. ramorum* zoospores

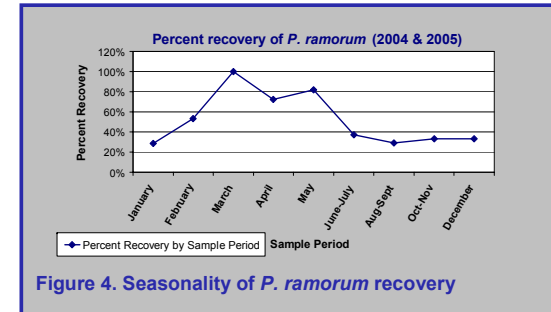


Figure 4. Seasonality of *P. ramorum* recovery

FUTURE WORK

- Identify other *Phytophthora* species occurring in stream courses with molecular analyses.
- Address research questions related to spread, survival, and quantification of *P. ramorum* in stream courses.
- Identify sources of inoculum and locations of infestation for new positive locations.

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