Ecology of *Phytophthora ramorum* in watercourses:

implications for the spread and management of Sudden Oak Death

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ABSTRACT

Though stream-baiting has proven useful for early detection of new terrestrial infestations of *Phytophthora ramorum*, the biological and ecological rationale behind the success of baiting are unknown. Our current studies address the central theme of “why baiting works,” focusing on specific questions including: i) what are the inoculum sources in streams?, ii) is there an inoculum threshold for baiting?, iii) can zoospore cysts undergo diplenetism to infect bait leaves?, iv) what are the trophic niches of *P. ramorum*. To address the potential for *P. ramorum* to persist on aquatic or riparian plants, several plant species were collected from a *P. ramorum*-infested pond in Humboldt Co., CA. Aboveground plant parts and roots were baited with rhododendron leaf disks; however, thus far, *P. ramorum* was not recovered from any plant tissues. Additionally, aquatic plants have been inoculated to determine their susceptibility to *P. ramorum*, but the pathogen has not been successfully re-isolated. To assess the role of trophic niche on bait detection of *P. ramorum*, living and dead rhododendron leaves were incubated in stream water (*in vitro* and *in vivo*). In streams, *P. ramorum* only colonized living baits; however, under laboratory conditions, *P. ramorum* colonized both living and dead baits. The inoculum threshold necessary for bait detection and the potential for cysts to undergo diplanetism (form zoospores) was investigated. The disparity between bait infectivity in the presence/absence of a surfactant suggests that cysts may diplanetise, and cysts were detected at concentrations as low as 10^2 cysts/ml by leaf disk baits.

Phytophthora ramorum- infested wetland in McKinleyville, CA

Sampling aquatic plants for *P. ramorum*

OBJECTIVES

1. Assess aquatic plants for infection by *P. ramorum* under natural conditions and subsequently determine susceptibility of these plants during controlled inoculations in the laboratory.
2. Determine whether *P. ramorum* infects living versus dead rhododendron leaf baits. Assess whether the pathogen may behave as a saprotroph in streams.
3. Investigate potential for diplanetism of zoospore cysts and the threshold of inoculum required for bait detection of cysts.
4. Assess the influence of turbulence on detection of cysts at low concentrations.

METHODS- OBJECTIVE 1

● A *P. ramorum*- infested wetland was identified in Humboldt Co, CA.

● Aquatic and riparian plants were collected and Individual plant parts (roots and shoots) were surface sterilized and baited with rhododendron leaf disks for detection of *P. ramorum*.

● Because *P. ramorum* was not detected on any plants collected, the same specimens were incubated in di water and inoculated with a zoospore suspension of *P. ramorum*.

● One week after inoculation, plant specimens were washed, surface sterilized and baited for determination of infection by *P. ramorum*.

Cattail (*Typha* sp.)

Cattail (*Typha* sp.)

Monkey flower (*Mimulus* sp.)

Duckweed (*Lemna minor*)

Red Alder (*Alnus rubra*)

RESULTS- OBJECTIVE 1

** *P. ramorum* was not found to infect any of the aquatic or riparian plants sampled**- including *Lemna minor*, *Mimulus sp*., *Ranunculus sp*., *Rumex obtusifolia*, *Typha sp*., *Veronica americana* & two unidentified grasses.

METHODS- OBJECTIVE 2

● Fresh, frozen, and oven-dried rhododendron leaves were maintained in mesh bags at three sampling points in streams flowing through one redwood and one mixed evergreen forest in Sonoma County for three week intervals, from February through April 2009.

● Upon retrieval, bait leaves were surface sterilized, evaluated for symptoms and isolations attempted on PARPH media from necrotic lesions or, in the absence of distinct lesions, from the tip, midrib and base of each leaf.

● The presence of *P.ramorum* or other Pythiacious species were determined by microscopic examination of cultures.

RESULTS – OBJECTIVE 2

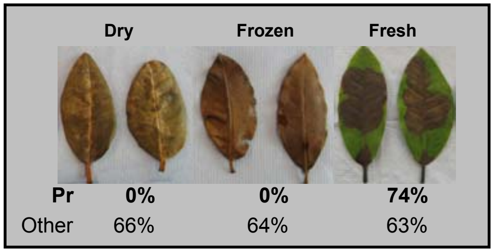


Figure 1. Percent of 90 total each of dry, frozen or fresh rhododendron leaves maintained in Sonoma County streams for three week intervals from late February through April 2009 infected with *P.ramorum* (Pr) or Other Pythiacious species.

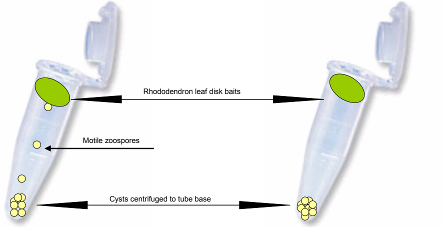
METHODS- OBJECTIVE 3

● Zoospore cysts of *P. ramorum* were suspended in 1 ml of di water in microcentrifuge tubes at the following concentrations: 0, 10^1, 10^2, 10^3, 10^4 cysts/ml.

● Cyst suspensions were centrifuged for 1 min at 12,000rpm to precipitate cysts on the bottom of tubes.

● A drop of surfactant (Tween) was added to half of the tubes, establishing a complete factorial treatment design including two surfactant treatments (+/- Tween) and 5 cyst concentrations.

● A rhododendron leaf disk was floated on the water surface in each tube and tubes were incubated upright. After 4 days, leaf disks were surface sterilized and placed on PARP for determination of infection.



No surfactant: cysts may germinate to motile zoospores and swim against chemotaxic gradient to infect bait.

Surfactant: membranes of motile zoospores will be disrupted by surfactant, preventing bait infection

RESULTS – OBJECTIVE 3

P. ramorum bait detection across cyst concentration gradient and in presence/absence of surfactant

Cyst Concentration (Cysts/ml)	Surfactant	No Surfactant
0	-	-
10^1	-	-
10^2	-	-
10^3	-	√
10^4	-	√

*No infection in presence of surfactant.

* Bait detection dependant on cyst concentration.

METHODS- OBJECTIVE 4

● To investigate role of diplanetism in turbulent and stable conditions in bait detection of *P. ramorum*, a complete factorial treatment design of 16 treatments included the following effects:

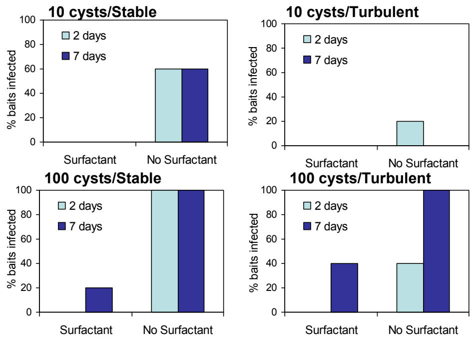
* 100 cysts vs. 10 cysts
* presence vs. absence of surfactant
* turbulent vs. sessile incubation
* 2 day vs. 7 day incubation

● Cysts were placed in 50 ml tubes filled with di water. A small rhododendron leaf was placed in each tube.

● Tubes were incubated either on a lab bench or on a shaker and were destructively sampled after either 2 days or 7 days incubation.

● Rhododendron leaves were surface sterilized, rinsed, cut into pieces, and placed on PARP to detect infection by *P. ramorum*.

RESULTS – OBJECTIVE 4



* Higher cyst concentrations, longer duration of bait incubation, and stable conditions are conducive to bait detection of *P. ramorum*.

* Presence of surfactant limited bait detection of *P. ramorum*.

CONCLUSIONS

● Thus far, *P. ramorum* has not been isolated from any of the aquatic plants sampled, nor has it been found infect these plants under controlled inoculations.

● In streams, *P. ramorum* behaves as a biotroph with respect to infection of rhododendron bait leaves.

● In vitro studies suggest that cysts of *P. ramorum* may diplentize and secondary zoospores may infect baits. Efficacy of bait detection of cysts is limited by low cyst concentration, short duration of bait incubation, and turbulence. We hypothesize that stable conditions promote a continuous chemotaxic gradient for zoospore homing.

ACKNOWLEDGEMENTS: Sarah Navarro, Akiko Oguchi, US Forest Service, Pacific Southwest Research Station