Status of California's *Phytophthora ramorum* Stream Survey

Kamyar Aram and **David M. Rizzo**,

University of California at Davis, Department of Plant Pathology, One Shields Avenue, Davis, CA

Overview:

Since 2004, we have annually monitored *Phytophthora ramorum* (Pr) in streams, creeks & rivers throughout infested and at-risk areas of California by baiting with rhododendron leaves. This survey helps delineate the pathogen's range and can quickly detects its spread. Our sites are dispersed from Del Norte to San Luis Obispo counties on the coast, and in certain counties of the Sierra Nevada foothills. Monitoring runs from February through June, the season of mild and moist conditions and thus highest pathogen activity.

Spread of Pr: In 2008 new detections of Pr occurred primarily adjacent to previously known infestation centers such as Redway, Humboldt County, the Navarro River watershed, Mendocino County, and the Big Sur in southern Monterey County. A new detection in the Little River at Van Damme SP marked the northern-most report of the pathogen in Mendocino county at the time, and was the most distant case from a previously confirmed occurrence. However, no terrestrial inoculum source has yet been identified. To date in 2009, we have detected Pr only in previously confirmed sites.

Seasonal trends: In 2008, the second relatively dry season in a row, the pathogen was cultured predominately from February and March samples. The most well-established Pr-infested sites (e.g. the Navarro River watershed) had the most consistent detection, while a few with previous detection were negative. In 2009, a season with more regular rainfall, we have detected the pathogen at most previously confirmed sites each month through April. This seasonal variation suggests that occurrence of Pr in streams coincides with periods of pathogen activity, and that detection in waterways during dry seasons may be diminished. Nonetheless, the pathogen's detection in both new and previously confirmed waterways indicate that this is a sensitive method for monitoring the spread of Pr.

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 whose assistance, permission, and guidance make this work possible.

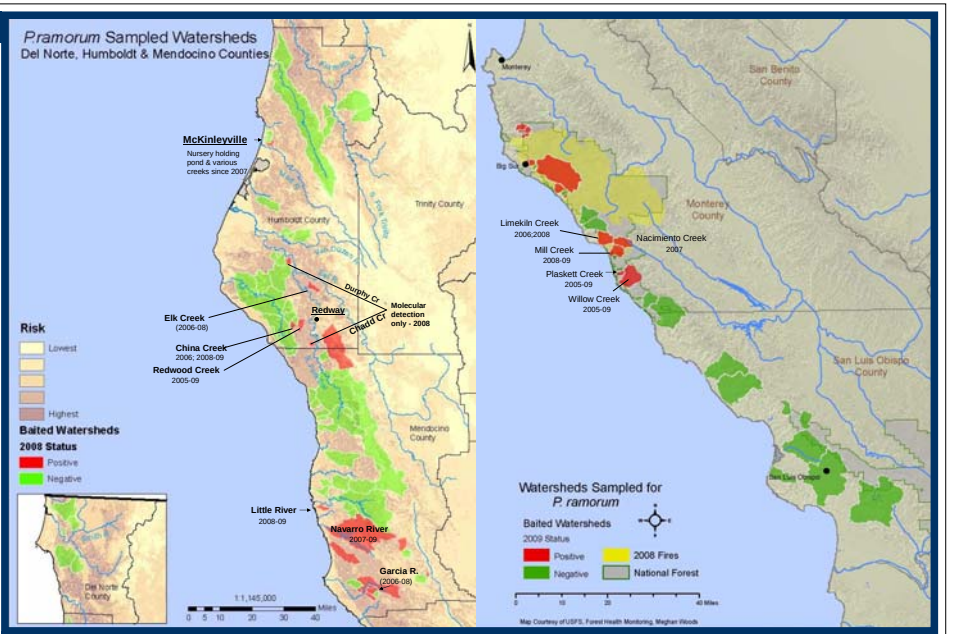
Methods:

Deployment:

- Five Rhododendron leaves of mature cuticle inserted into pockets sown into 1mm fiberglass mesh bags.
- Two bags per site secured separately by rope to banks of streams and floated near the water surface.
- Baits exposed approximately 21, 14 and 10 days, while temperatures are below 12, 12-15, or above 15ºC, respectively, with collector discretion for maximum bait exposure without degradation.

Evaluation:

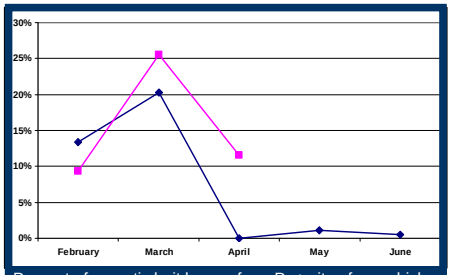
- Leaves surface sterilized in 1% bleach solution for 1-2 minutes and rinsed thoroughly with DI water.
- Each leaf categorized as lesion free, partially or wholly necrotic, or dead (cuticle degraded).
- Margins of necrosis isolated onto PARP with 0.025g/L hymexazol. When no margin is discernible (i.e. lesion free or completely necrotic), samples taken from leaf tip, midrib, and base.
- Cultures incubated at 18°C for three weeks and regularly examined with a microscope for growth of Pr and other Phytophthora species.
- In certain cases, results are checked again by DNA analysis for presence of Pr.



Some watersheds where Pr has been detected by baiting (in red) are listed with the years of positive samples below the name. Years in parentheses indicate sites not being monitored in 2009.

Key observations:

- Pr predominately spread to adjacent watersheds.
- No terrestrial inoculum source determined for the Little River or in creeks around McKinleyville.
- Chadd & Durphy Creeks in Humboldt County Pr + by DNA analysis only in 2008; no culture detection to date, April 2009.
- Pr primarily detected early in the season in 2008.
- 2009 detections suggest longer period of Pr activity than in 2008.
- Overall, Pr observed on less than 5% of lesion free or dead leaves; Bait leaves with necrotic lesions account for most successful isolations (Table 1).



Percent of necrotic bait leaves from Pr + sites from which the pathogen was cultured in 2008 (blue) and 2009 (red).

Table 1. Association of bait condition – lesion free, necrotic (partially or wholly), or dead (cuticle degraded) - with successful Pr isolation for Pr + sites only.

	% of Pr + baits	% of all baits collected
Lesion Free	1	9
Necrotic	96	72
Dead	3	19

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Rizzo Lab, UC Davis Plant Pathology:
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