

**2009 *Phytophthora ramorum* Survey
for the Northern Sierra Nevada
California Department of Forestry and Fire Protection
Submitted by Donald R. Owen
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Summary

A stream-based detection survey was conducted in Butte, Yuba, Nevada, Placer, and El Dorado Counties in northern California for *Phytophthora ramorum*, the cause of sudden oak death. A total of 20 watercourse sites were surveyed during May and June using rhododendron leaves as bait for *Phytophthora* spp. This produced 39 sets of baits that were cultured for the presence of *P. ramorum*. The pathogen was not detected in any of the samples. Other *Phytophthora* spp. were recovered but not identified.

SUDDEN OAK DEATH / *P. ramorum* SURVEY

The quarantined disease organism *P. ramorum* is not established in the Sierra Nevada of California, although hosts and putatively suitable habitat occur there. Risk analyses (Meentemeyer et al. 2004*) identified the foothills of the northern Sierra Nevada, from El Dorado County north to Butte County, as having the most suitable habitat for establishment of *P. ramorum* within the mountain range. This is the 6th year that some portion of this area has been surveyed for *P. ramorum*. This year's objectives were to 1) use rhododendron baits to recover *Phytophthora* spp. from principal watercourses draining these habitats and 2) conduct follow-up surveys if *P. ramorum* was recovered.

The project was planned and coordinated by CDF Entomologist Don Owen. Retired USDA Forest Service Silviculturalist Karen Jones-Schimke was the surveyor. Surveys were conducted from early May through late June 2009. Kamyar Aram (Dave Rizzo's Lab, UC Davis) provided the baits and conducted the diagnostics for watercourse samples. Surveys were conducted on private land, portions of the Plumas, Tahoe and Eldorado National Forests, and several State Recreation Areas. Personnel from the Yuba River Ranger District provided transportation across New Bullard's Bar Reservoir and assisted with surveys at remote watercourse sites there.

Procedures

In 2006, Douglas Shoemaker of the Center of Applied GIS (under the direction of Ross Meentemeyer), University of North Carolina, provided 1:100,000 scale maps delineating areas of moderate to high risk for the establishment of *P. ramorum* in the northern

* Meentemeyer, R, D Rizzo, W Mark, and E Lotz. 2004. Mapping the risk of establishment and spread of sudden oak death in California. For Ecol and Mgmt. 200: 195–214.

Sierra Nevada. These were overlaid with standard 1:100,000 USGS maps to identify access roads and watercourses that traverse high-risk habitats. Locations of potential watercourse-sampling sites were identified and plotted on 1:24,000 scale topographic maps and 1:12,000 orthophotos for field use. Following a reconnaissance of potential sites, a total of 23 watercourses were sampled in 2006 in Butte, Yuba, Nevada, Placer, and El Dorado Counties. A number of sites were moved or added in 2007 and 2008, resulting in 28 and 31 watercourse sites, respectively, being sampled during these years. In 2009, due to an increased work load, Kamyar Aram requested that the number of sites be limited to 20.

Watercourses were sampled using a slight modification of the UC Davis – Rizzo Lab sampling protocol (Attachment at end), which utilizes *Rhododendron* leaves as “bait” for *Phytophthora* spp. Each watercourse was sampled over two sequential time periods of approximately 2-3 weeks each, with 1 replicate/watercourse site for each sampling period (a total of 2 sets of baits for each watercourse site). Placement of baits began May 5th and the last baits were retrieved June 28 (see Table at end of report). Most watercourses were accessed by road or trail, but some watercourses entering New Bullard’s Bar Reservoir were accessed by boat. Samples were processed at UC Davis to determine if *Phytophthora ramorum* was present. Incidental to this, other *Phytophthora* spp., were often recovered.

Results and Discussion

Twenty watercourse sites were baited. Ultimately, a total of 39 sets of baits (see Table) were submitted for diagnostics. Watercourses were located in the following major drainages: Feather, Yuba, Bear, American, and Consumnes Rivers. *Phytophthora ramorum* was not recovered from any of the watercourses.

Over the past 6 years, the following surveys have been conducted in the northern Sierra Nevada:

- 2004: 23 vegetation transects in Tehama and Butte Counties
- 2005: 35 vegetation transects and 11 watercourse sites baited in Butte and Yuba Counties
- 2006: 32 vegetation transects and 23 watercourse sites baited in Butte, Yuba, Nevada, Placer, and El Dorado Counties
- 2007: 28 watercourse sites baited in Butte, Yuba, Nevada, Placer, and El Dorado Counties
- 2008: 31 watercourse sites baited in Butte, Yuba, Nevada, Placer, and El Dorado Counties
- 2009: 20 watercourse sites baited in Butte, Yuba, Nevada, Placer, and El Dorado Counties

P. ramorum has not been detected by these surveys, however, other *Phytophthora spp.* commonly found in forest environments have been recovered from both vegetation and watercourse samples. Also, hosts for *P. ramorum* are abundant in all areas surveyed. These results support the utility of the survey methods and indicate that *P. ramorum* likely could become established in this area if it were introduced into a suitable local environment.

Location of Watercourse Sites and Sampling dates. California 2009.

Site Name	UTM (WGS 84)		County	1 st		2 nd	
	easting	northing		Sample date out	date in	Sample date out	date in
Frey Creek	648452	4387332	Butte	19-May	2-Jun	16-Jun	28-Jun
French Creek	638938	4397494	Butte	21-May	3-Jun	16-Jun	28-Jun
Flea Creek	632493	4407182	Butte	5-May	21-May	4-Jun	18-Jun
Mill Creek (Butte Co)	633770	4407182	Butte	5-May	21-May	4-Jun	18-Jun
Bridger Creek	664071	4369303	Yuba	18-May	4-Jun	18-Jun	28-Jun
Little Oregon Cr	657085	4365909	Yuba	7-May	20-May	3-Jun	17-Jun
Middle Fork Yuba R	664885	4362007	Yuba/Nevada	7-May	20-May	3-Jun	17-Jun
Indian Creek	657583	4371431	Yuba	18-May	4-Jun	18-Jun	28-Jun
Deer Creek	671512	4347885	Nevada	7-May	26-May	9-Jun	23-Jun
Steephollow Ck.	681806	4338551	Nevada	13-May	26-May	9-Jun	23-Jun
Wolf Ck	663860	4323753	Nevada	11-May	26-May	9-Jun	23-Jun
S. Fork of the Yuba	663890	4351087	Nevada	11-May	baits lost	20-May	3-Jun
N Fk of Consumnes	699991	4280824	EIDorado	14-May	28-May	11-Jun	25-Jun
Rock Creek	693140	4296423	EIDorado	14-May	28-May	11-Jun	25-Jun
Weber Creek	694496	4286674	EIDorado	14-May	28-May	11-Jun	25-Jun
N. Fork of American	678315	4318730	Placer	12-May	27-May	10-Jun	24-Jun
N Fk of Mid Fk of Am	697787	4322342	EIDorado	12-May	27-May	10-Jun	24-Jun
Rubicon River	697107	4319271	EIDorado	12-May	27-May	10-Jun	24-Jun
S. Fk. Am. River	692368	4293048	EIDorado	14-May	28-May	11-Jun	25-Jun
M. Fk. Am. River	696396	4319846	Placer	12-May	27-May	10-Jun	24-Jun

End of Report - **Attachment follows**

Attachment

UC DAVIS-RIZZO LAB WATERCOURSE BAIT METHODS

Watercourse selection:

- Sites are selected based on accessibility, local cooperation (for remote locations), minimum visibility, broadly representing county watercourses, and perennial water flow
- All watershed and watercourse sizes are considered (within reason) although accessibility during floods can be limited; we have recovered *P. ramorum* from one large river
- Each site is sampled at six week intervals year-round; adjusting sample location, time of leaves in watercourse, and replacing parts as needed
- GPS coordinates of each site are recorded to use for mapping

Bait placement:

- Make baiting bags out of approx 1mm fiberglass mesh (window screening material); cut square foot pieces and fold one edge back toward other edge, leaving approx 4" of non-overlap, and staple edges; staple five equal size pockets along the width of bag; make sure enough overlap of extra mesh to cover openings of pockets
- Clean, disease-free Rhododendron (we use Colonel Cohen horticultural variety, Gomer waterii variety also works well but any will work) leaves are placed in mesh bags
- Place bubble wrap at end slots in bags to help float bag near water surface
- Weave rope (nylon 3/16") through mesh bag to hold flap closed
- Ten leaves are placed at each location with two replicate locations per site.
- Bags are secured to riverbanks and floated near the water surface for 7-21 days with the minimum time period in warm weather and warm watercourse temperatures and longer intervals in cold conditions. Interval time adjusted year-round.
- Tie bag up high on riverbank to secure location (preferably so location is accessible during all flood stages)
- Consider attachment of 1 lb round fishing weight with highly visible and heavy gauge fishing line or use large rocks if needed to keep bag in regular watercourse flow and away from edge/bank
- Flag rope with contact info
- Clean soil/mud off boots used for accessing watercourse (rubber boots work great)- use 95% Ethanol or 10% bleach water; optional if not infested watercourse course

Collection:

- Remove leaves from water and place in separate sample collection bags
- Rinse bag and leaves in watercourse if dirt and detritus on leaves/bag/rope
- Take water temperature of watercourse at pick up- leave thermometer in water +2min (this helps evaluate how long to leave baits in watercourses)
- Sterilize removed bags in 10% bleach water for 20-30 minutes, rinse, and dry; reuse on future sampling
- Refrigerate samples prior to isolation

Isolations:

- Leaves are surface sterilized in 95% Ethanol for 30 seconds, rinsed with DI water, and air-dried for 1-2 hours. (Optional- Hansen Lab does not do this step, alternatively they just clean leaves with DI water, I like to make sure infection is on that leaf and not cross-contamination from other leaves in sample bag)

- Disease symptoms are described and recorded for all leaves.
- Symptomatic leaves are isolated onto *Phytophthora*-selective media (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). Current experiments are being conducted examining hymexazol inhibition on other *Phytophthora* species. Hansen lab also uses this media.
- Submerge 10-15 leaf pieces max in media per petri-plate as flat as possible (in order to see structures forming around leaf surface) and to permit space for hyphal growth and clean transfer of organism
- Plates are incubated at 18°C

Results:

- Check plates every three to five days microscopically, carefully examining each leaf piece around entire edge for hyphae and/or reproductive structures
- Keep plates at least 3-4 weeks for late recovery of pathogens
- Any *Phytophthora*-like organisms are transferred and further examined for identification.