

**2010 *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 22 stream sites were sampled during May, June, and the beginning of July using rhododendron leaves as bait for *Phytophthora* spp. Eighty-six sets of baits were evaluated and/or 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 7th year that some portion of this area has been surveyed for *P. ramorum*. Objectives were to 1) use rhododendron baits to recover *Phytophthora* spp. from principal streams draining these habitats and 2) conduct follow-up surveys if *P. ramorum* is recovered. The recovery of *P. ramorum* from a stream would indicate the establishment of the pathogen within the drainage upstream from the location of the sample.

The project was planned and coordinated by California Department of Forestry and Fire Protection Entomologist Don Owen. Retired USDA Forest Service Silviculturalist Karen Jones-Schimke conducted the survey. Stream sampling began in early May and ended early July 2010. Kamyar Aram (Dave Rizzo's Lab, UC Davis) provided the baits and conducted the diagnostics for stream samples. Sampling was conducted on private land, portions of the Plumas, Tahoe and Eldorado National Forests, and in State Recreation Areas. Personnel from the Yuba River Ranger District provided transportation across New Bullard's Bar Reservoir and assisted with surveys 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

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\* 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.

areas of moderate to high risk for the establishment of *P. ramorum* in the northern Sierra Nevada. These were overlaid with standard 1:100,000 USGS maps to identify access roads and streams that traverse high-risk habitats. Locations of potential 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 stream sites 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 sites, respectively, being sampled during those years. Because of a limit on the number of samples that could be processed at the UC diagnostics lab, 20 sites were sampled in 2009 and 22 sites this year.

Streams 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 stream site was sampled continuously using one set of baits (5 leaves) over the duration of approximately 8 weeks. Baits were changed approximately every 2 weeks, so there were 4 sampling periods and 4 sets of baits deployed per site. Placement of baits began May 4th and the last baits were retrieved July 7 (see Table at end of report). Most streams were accessed by road or trail, but some streams 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

A total of 86 sets of baits were submitted for diagnostics (2 sets of baits were lost to flooding). Sampling was done in the following major drainages: Feather, Yuba, Bear, and American Rivers. *Phytophthora ramorum* was not recovered from any of the stream sites.

Over the past 7 years, the following surveys have been conducted in the northern Sierra Nevada:

- 2004: 23 vegetation transects in Tehama and Butte Cos.
- 2005: 35 vegetation transects and 11 stream sites baited in Butte and Yuba Cos.
- 2006: 32 vegetation transects and 23 stream sites baited in Butte, Yuba, Nevada, Placer, and El Dorado Cos.
- 2007: 28 stream sites baited in Butte, Yuba, Nevada, Placer, and El Dorado Cos.
- 2008: 31 stream sites baited in Butte, Yuba, Nevada, Placer, and El Dorado Cos.
- 2009: 20 stream sites baited in Butte, Yuba, Nevada, Placer, and El Dorado Cos.
- 2010: 22 stream sites baited in Butte, Yuba, Nevada, Placer, and El Dorado Cos.

*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 2010.

<u>Site Name</u>	<u>UTM (WGS 84)</u>		<u>County</u>	<u>date out</u>	<u>baits exchanged</u>	<u>baits exchanged</u>	<u>baits exchanged</u>	<u>date in</u>
	<u>E</u>	<u>N</u>						
Frey Creek	648452	4387332	Butte	13-May	26-May	1-Jun	15-Jun	29-Jun
French Creek	638938	4397494	Butte	13-May	26-May	1-Jun	15-Jun	29-Jun
Chino Creek	636780	4399410	Butte	13-May	26-May	1-Jun	15-Jun	29-Jun
Galen Creek	637432	4390490	Butte	13-May	26-May	1-Jun	15-Jun	29-Jun
Little Oregon Cr.	657064	4365908	Yuba	5-May	19-May	8-Jun	22-Jun	28-Jun
Costa Creek	651369	4372376	Yuba	5-May	19-May	8-Jun	22-Jun	28-Jun
South Yuba R.	655847	4350849	Nevada	5-May	19-May	8-Jun	22-Jun	28-Jun
Middle Yuba R.	664885	4362007	Yuba/Nevada	5-May	19-May	8-Jun	22-Jun	28-Jun
Sucker Run	645898	4379562	Butte	5-May	19-May	8-Jun	22-Jun	28-Jun
South Feather R.	647801	4379379	Butte	5-May	19-May	8-Jun	22-Jun	28-Jun
Burnt Bridge Cr.	658267	4365226	Yuba	12-May	26-May	7-Jun	--	6-Jul
Indian Creek	657583	4371431	Yuba	12-May	26-May	7-Jun	--	6-Jul
Deer Creek	671512	4347885	Nevada	4-May	18-May	1-Jun	17-Jun	28-Jun
Bear River @ Dog Bar	672514	4325521	Nevada/Placer	4-May	18-May	1-Jun	17-Jun	28-Jun
Bear R. @ Steepollow Cr.	681806	4338551	Nevada/Placer	4-May	18-May	1-Jun	17-Jun	28-Jun
Wolf Creek	663860	4323753	Nevada	4-May	18-May	1-Jun	17-Jun	28-Jun
Rock Creek	693140	4296423	EIDorado	17-May	27-May	9-Jun	24-Jun	7-Jul
NF American R.	678315	4318730	Placer	17-May	27-May	9-Jun	24-Jun	7-Jul
NF MF American R.	697787	4322342	EIDorado	17-May	27-May	9-Jun	24-Jun	7-Jul
Rubicon River	697107	4319271	EIDorado	17-May	27-May	9-Jun	24-Jun	7-Jul
SF American R.	693036	4295024	EIDorado	17-May	27-May	9-Jun	24-Jun	7-Jul
MF American R.	679333	4314629	Placer	17-May	27-May	9-Jun	24-Jun	7-Jul

## **Errata**

Incorrect coordinates were given for two of the stream sites in the 2009 Report. The correct UTM coordinates (WGS 84) are:

French Creek	E 638938	N 4397494
S. Fork American R.	E 692368	N 4293048

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.