

**2007 *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 28 watercourse sites were surveyed during May, June, and July using rhododendron leaves as bait for *Phytophthora* spp. This produced 96 sets of baits that were cultured for the presence of *P. ramorum*. The pathogen was not detected in any of the samples. *Phytophthora gonapodyides*, a common inhabitant of western forests, was recovered from a number of watercourses.

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 (Ross Meentemeyer, UNC Charlotte) 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 4th year that some portion of this area has been surveyed for *P. ramorum* as part of the National *P. ramorum* Wildland Survey. This year's objectives were to 1) use rhododendron baits to recover *Phytophthora* spp. from the principal watercourses draining these habitats, 2) to expand and further refine the watercourse sampling sites within the area, and 3) conduct follow-up surveys if *P. ramorum* was recovered.

The project was planned and coordinated by CDF Entomologist Don Owen. Retired CDF Forester Don MacKenzie was the principal surveyor. Surveys were conducted from late May through early July 2007. Shannon Murphy (Dave Rizzo's Lab, UC Davis) provided the protocol, 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

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. The principal criteria for site selection are the amount of high risk habitat that exists upstream of a site and site access. To better address these criteria, a number of sites were moved or added in 2007, resulting in a total of 28 watercourse sites being sampled this year.

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 2 replicates/watercourse site for each sampling period (a total of 4 sets of baits for each watercourse site, provided no baits were lost). Placement of baits began May 25 and the last baits were retrieved July 4 (Table 1). 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

Thirty watercourse sites were initially baited. Ultimately, a total of 96 sets of baits, collected from 28 watercourse sites (Table 1), were submitted for diagnostics. The 28 sites were distributed among 27 separate watercourses, with the main stem of the Bear River having 2 sites. 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. *Phytophthora gonapodyides*, a common inhabitant of western forests*, was recovered from Pilot, Camp, Berry, French, Rock, Deer, and Willow Creeks, and the lower Bear River (Dog Bar). In 2006, *P. gonapodyides* was recovered from Bridger, Frey, Stepphollow, Wolf, Deep Canyon, and Rock Creeks, and the Lower Bear River.

A principal difference between this year and the previous 2 years was the amount of spring precipitation. In 2005, the cumulative total inches of precipitation for the months of March, April, and May averaged for 8 northern Sierra weather stations was 21.1 inches; in 2006 it was 28.1 inches; and in 2007 it was 6.0 inches -- less than a third of either of the previous 2 years (California Department of Water Resources). It is presumed that spring precipitation could influence the recovery of *Phytophthora*

* Hansen, EM, p. 23-27 IN *Phytophthora* Diseases of Forest Trees: Proceedings from the first International Meeting on Pythophthoras in Forest and Wildland Ecosystems, Grants Pass, OR. Oregon State Univ. (2000).

ramorum from watercourses in the northern Sierra Nevada, but there is no direct evidence of this since the pathogen has never been isolated from this part of the State.

Over the past 4 years, the following surveys have been conducted in the northern Sierra Nevada under the auspices of The National Wildland Survey for *P. ramorum*:

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

Challenges encountered include:

- difficult terrain, access, and a huge land base
- a multitude of water impoundments throughout the survey area that serve as 'dead-ends' for downstream transport of *Phytophthora* propagules; hence, impoundments limit the amount of upstream habitat that is effectively surveyed by baits.
- high water levels and debris during spring runoff, especially in wet years
- dry spring weather, which potentially could limit sporulation by *Phytophthora ramorum*.

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.

End of Report

Tables and Attachment follow

Table 1. Location of Watercourse Sites and Sampling dates. California 2007.

Site Name	UTM E	(WGS84) N	date out	date in	date out	date in	County
Sucker Run	645854	4379459	6/6/07	6/23/07	6/23/07	7/4/07	Butte
Frey Creek	648459	4387317	6/6/07	6/23/07	6/23/07	7/4/07	Butte
French Creek	639415	4396036	6/8/07	6/23/07	6/23/07	7/4/07	Butte
Berry Creek	634509	4391446	5/31/07	6/21/07	6/21/07	7/1/07	Butte
Flea Valley	632493	4407182	5/31/07	6/21/07	6/21/07	7/1/07	Butte
Mill Creek (Butte Co)	633770	4407182	5/31/07	6/21/07	6/21/07	7/1/07	Butte
Willow Creek	665649	4368811	5/30/07	6/14/07	6/14/07	6/28/07	Yuba
Upper Indian Creek	656290	4371849	6/9/07	6/28/07	---	---	Yuba
Little Oregon Cr	657085	4365909	5/30/07	6/14/07	6/14/07	6/28/07	Yuba
Middle Fork Yuba R	664885	4362007	5/30/07	6/14/07	6/14/07	6/24/07	Yuba / Nevada
Mill Creek (Yuba Co)	660977	4368406	6/18/07	7/3/07	---	---	Yuba
Indian Creek	657583	4371431	6/18/07	7/3/07	---	---	Yuba
Wolf Ck Grass Valley	666656	4341587	5/25/07	6/11/07	6/11/07	6/24/07	Nevada
Deer Creek	671512	4347885	5/25/07	6/11/07	6/11/07	6/25/07	Nevada
Upper Bear River	681827	4338584	---	---	6/12/07	7/2/07	Nevada / Placer
Willow Creek	673847	4331415	5/27/07	6/11/07	6/11/07	6/24/07	Nevada
Lower Bear River	664152	4320009	5/27/07	6/11/07	6/11/07	6/22/07	Nevada / Placer
Wolf Ck Alta Sierra	669127	4330069	6/4/07	6/11/07	6/11/07	6/25/07	Nevada
Kentucky Creek	655343	4350553	5/25/07	6/11/07	6/11/07	6/24/07	Nevada
Squirrel Creek	656554	4340795	6/7/07	6/24/07	6/22/07	7/2/07	Nevada
Dog Bar	672514	4325521	6/12/07	6/24/07	---	---	Nevada
N Fk of Mid Fk of Am	695321	4320160	5/29/07	6/13/07	6/13/07	6/26/07	Placer Placer / El
Rubicon River	697107	4319271	5/29/07	6/13/07	6/13/07	6/26/07	Dorado
Pilot Creek	702370	4312275	6/4/07	6/20/07	6/20/07	6/30/07	El Dorado
N Fk of Consumnes	699991	4280824	6/1/07	6/15/07	6/15/07	6/27/07	El Dorado
Rock Creek	693140	4296423	6/4/07	6/20/07	6/20/07	6/30/07	El Dorado
Weber Creek	698655	4287495	6/1/07	6/15/07	6/15/07	6/27/07	El Dorado
S. Fk. Am. River	689221	4293039	6/20/07	6/30/07	---	---	El Dorado

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.