California 2006 National *Phytophthora ramorum* Wildland Survey
California Department of Forestry and Fire Protection

Submitted by Donald R. Owen
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Summary

As part of the 2006 National *Phytophthora ramorum* wildland survey, detection surveys were conducted in Butte, Yuba, Nevada, Placer, and El Dorado Counties in northern California. Two types of surveys were conducted: 1) a road survey combined with vegetation transects to record hosts of *P. ramorum* and sample symptomatic host tissue, and 2) a stream survey that utilized rhododendron leaves as bait for *Phytophthora* spp. in stream water. A total of 32 vegetation transects were surveyed and 23 streams baited. Additionally, hundreds of miles of roadside vegetation were scanned while driving through areas identified as moderate to high risk for sudden oak death. Four vegetation samples and 85 aquatic samples were collected for lab diagnosis. *P. ramorum* was not detected by any of the survey methods. Other *Phytophthora* species were baited from a number of streams, including the commonly encountered *P. gonapodyides*.

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 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. The objectives of this year's surveys were to 1) survey for the presence and/or absence of *P. ramorum* on plant species in moderate to high-risk wildland habitats that are *not* known to be infested in the northern Sierra Nevada, 2) use rhododendron baits to recover *Phytophthora spp.* from the principal streams draining these habitats, 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 early May through July 2006. Cooperators included Ross Meentemeyer of the University of North Carolina at Charlotte, who produced risk maps for *P. ramorum*, and David Rizzo of UC Davis and Matteo Garbelotto of UC Berkeley, whose labs performed diagnostics on samples. Shannon Murphy (Rizzo Lab, UC Davis) provided the protocol, baits, and conducted the diagnostics for stream samples. Surveys were conducted on private land, portions of the Plumas, Tahoe and Eldorado National Forests, and several State Recreation Areas. Jerry Cowan of the Yuba River Ranger District provided transportation

across New Bullard's Bar Reservoir and assisted with surveys at remote stream sites there.

Procedures

Douglas Shoemaker of the Center of Applied GIS, 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 streams that traverse high-risk habitats. Locations of potential vegetation transects and stream-sampling sites were identified and plotted on 1:24,000 scale topographic maps and 1:12,000 orthophotos for field use. A reconnaissance was made to determine accessibility. In 2005, the survey was conducted in high risk habitats of Butte and Yuba Counties. This year's survey again sampled streams in these counties and expanded south to include streams in Nevada, Placer, and El Dorado Counties, for a total of 23 streams sampled. A total of 32 vegetation transects were surveyed, mostly in Nevada, Placer, and El Dorado Counties.

Streams were sampled using the protocol of the UC Davis – Rizzo Lab (Attachment at end), which utilizes Rhododendron leaves as "bait" for *Phytophthora* spp. Each stream was sampled over two sequential time periods of approximately 3 weeks each, with 2 sampling sites/stream for each sampling period (a total of 4 sets of baits for each stream, provided no baits were lost). Baiting was initiated May 9th and ended July 7th (Table 1). Most streams were accessed by road, but streams entering New Bullard's Bar Reservoir were accessed by boat. Samples were processed at UC Davis to determine which *Phytophthora spp.*, if any, were present.

Vegetation transects were surveyed using a slightly modified version of the protocol of the **National *Phytophthora ramorum* Survey of Forest Environments** (http://fhm.fs.fed.us/sp/sod/ndsurvey/06/methods/early_detection.pdf). The National protocol utilizes four transects of 100 m that follow cardinal directions from a plot center. The steep ground throughout much of the survey area made this impractical and, in some cases, unsafe. Slopes in excess of 40% were common and some slopes approached 100%. Also, much of the survey was on private land where access was restricted. We used single transects of 400 m or more that followed roads and trails. Host plants were examined for symptoms associated with *P.* ramorum infection along the length of the transect and the presence of all host species/genera were recorded. We also conducted windshield surveys of roadside vegetation as we drove between transect locations. Samples for lab diagnosis were collected whenever symptoms of *P. ramorum* were encountered. Samples were shipped to the Garbelotto Lab for PCR diagnostics and to the Rizzo Lab for culturing. Samples were labeled with host species, transect number, UTM coordinates, and collection date. Procedures for handling and

processing samples and reporting results are outlined in the protocol. The center point of each transect was recorded by GPS in UTM NAD 83 Coordinates.

Results and Discussion

Twenty-five streams were initially baited, but two streams entering New Bullards Bar Reservoir were dropped because of difficult access. Ultimately, a total of 85 sets of baits, collected from 23 streams (Table 1), were submitted for diagnostics. Streams were located in the following major drainages: Feather, Yuba, Bear, American, and Consumnes Rivers. *Phytophthora ramorum* was not recovered from any of the streams. *Phytophthora gonapodyides,* a common inhabitant of western forests*, was recovered from Bridger, Frey, Steephollow, Wolf, Deep Canyon, and Rock Creeks, and the Lower Bear River. Challenges included difficult access and rising water levels and debris during spring runoff. Also, the multitude of water impoundments throughout the survey area are considered to be 'dead-ends' for downstream transport of *Phytophthora* propagules and, hence, impoundments sometimes limited the amount of upstream habitat that was effectively surveyed by baits.

Thirty-two transects were walked and visually scanned for hosts and symptoms of *P. ramorum* infection (Table 2). Twenty hosts of *P. ramorum* were recorded (Table 3). The most common host was black oak, found on all but 5 transects. Other common hosts were canyon live oak, toyon, poison oak, madrone, big leaf maple, manzanita, California buckeye, and California bay laurel, all of which were found on half or more of the transects. Bay laurel was found on 16 transects, while last year it was found on only 4 transects. This may in part be a reflection of accessibility. Areas surveyed this year were more developed and thus had greater accessibility than areas surveyed in 2005. Tanoak was found on 4 transects, while last year it was found on all but one transect. This result generally fits with the distribution of tanoak in the Sierra Nevada as reported by Griffin and Critchfield (1972. The distribution of forest trees in California. USDA For. Serv. Res. Pap. PSW-82), which shows the greatest concentration of tanoak in Butte and Yuba Counties, while Nevada, Placer, and El Dorado Counties have relatively modest amounts.

Despite the large number of hosts encountered during the survey, few symptoms of disease were found. Four vegetation samples (2 tanoak, 1 bay, and 1 toyon) were collected on transects and submitted for diagnostics. None had compelling symptoms and all were negative for *Phytophthora* infection.

For the past 2 years, the survey has focused on habitats suitable for the establishment

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

of *P. ramorum* in Butte, Yuba, Nevada, Placer, and El Dorado Counties. This area is roughly a thousand square miles in size. The numbers of hosts recorded by the survey, both this year and last, clearly confirms that host presence would not be a limiting factor for the establishment of *P. ramorum* in the northern Sierra Nevada. Bay and/or tanoak were recorded on 19 of 32 transects in 2006. The 2005 survey recorded one and/or the other of these hosts on 34 of 35 transects.

A total of 131 aquatic and vegetation samples were collected and submitted for diagnostics during 2005 and 2006. Similarly, hundreds of miles of roadside vegetation were visually scanned for symptoms of sudden oak death. As such, California's National Wildland Survey has made a best effort to detect *P. ramorum* within suitable and reasonably accessible habitats of the northern Sierra Nevada over the past 2 years. It is the only survey that currently targets SOD in this part of the State and, therefore, is an important component of the overall effort to monitor and detect new occurrences of SOD in California. The survey also provides valuable input to University of California research on monitoring *P. ramorum* and other *Phytophthora spp.* in streams. Continuation of the Survey is recommended for these reasons.

End of Report
Tables and Attachment follow

Table 1. **Location of Stream Sites and Sampling dates.** California 2006.

Stream Name	UTM E	N	date out	date in	date out	date in
Mill Cr (Butte Co)	633772	4407177	5/10/06	5/31/06	5/31/06	6/15/06
Bridger Cr	664071	4369303	5/9/06		6/10/06	7/7/06
Little Oregon Cr	657064	4365908	5/9/06	6/3/06	6/3/06	6/16/06
Middle Fork Yuba R	664909	4361990	5/9/06	6/3/06	6/3/06	6/16/06
Sucker Run	645871	4379591	5/23/06	6/13/06	6/13/06	6/30/06
Frey Creek	648452	4387332	5/10/06	5/31/06	5/31/06	6/15/06
French Cr	637643	4395310	5/11/06		6/1/06	6/27/06
French Cr	640039	4398318	6/13/06	6/27/06		
Flea Valley Cr	632495	4407257	5/10/06	5/31/06	5/31/06	6/13/06
Berry Cr	634509	4391445	5/11/06	6/1/06	6/1/06	6/16/06
Cascade Canal	678727	4348493	5/15/06	6/2/06	6/2/06	6/20/06
Deer Cr	671183	4347765	6/3/06	6/20/06		
Tarr Ditch	655652	4337449	6/3/06	6/12/06	6/15/06	7/5/06
Steephollow Cr	682470	4340558	5/16/06	6/5/06	6/5/06	6/20/06
Willow Cr	673847	4331415	5/23/06	6/3/06	6/3/06	6/20/06
Lower Bear R	672514	4325521	5/16/06	6/5/06	6/5/06	6/20/06
Wolf Cr	669127	4330069	5/16/06	6/5/06	6/5/06	6/20/06
Kentucky Cr	655343	4350553	5/17/06	6/2/06	6/2/06	6/20/06
Weber Cr	690184	4286687	5/18/06	6/8/06	6/8/06	6/22/06
Deep Canyon Cr	702000	4310500	5/12/06	6/7/06	6/7/06	6/21/06
Mid. Fk. Am. R	695158	4320288	5/18/06	6/11/06	6/11/06	
N Fk of Mid Fk of Am R	695203	4320363	5/18/06	6/11/06	6/11/06	6/21/06
N Fk of Consumnes R	699991	4280824	5/23/06	6/9/06	6/9/06	6/22/06
Rock Cr	693149	4296394	5/23/06	6/9/06	6/9/06	6/22/06
		not recovered				not baited

Table 2. Locations and dates of Vegetation Transects. California 2006.

Site ID	Date	Location	UTM Easting	Northing	County	Bay	Tanoak
06-01	6-Jun	Birchville	662500	4354500	Nevada		
06-02	6-Jun	Sweetland	662000	4358000	Nevada		
06-03	26-May	S Yuba SP	655500	4351000	Nevada	X	
06-04	26-May	S Yuba SP	656298	4350916	Nevada		
06-05	11-May	Berry Creek	635000	4391000	Butte	X	X
06-06	5-Jun	Lowell Hill Rd	682470	4340558	Nevada		X
06-07	6-Jun	Independence Tr	664068	4350862	Nevada	X	
06-08	7-Jun	Auburn Rec A	670369	4308832	ElDorado	X	
06-09	7-Jun	Deep Cyn Ck	702070	4310485	ElDorado		X
06-10	9-Jun	Newtown Rd	701500	4286320	ElDorado		
06-11	20-Jun	Pasquale Rd	678727	4348493	Nevada		X
06-12	26-Jun	Indian Sps Ck	657040	4334403	Nevada		
06-13	5-Jul	Gateway Park	656252	4341000	Nevada		
06-14	5-Jul	North Star	666646	434174	Nevada		
06-15	5-Jul	Robie Point	669112	4306580	Nevada	X	
06-16	8-Jul	Rex Reservoir	660317	4343262	Nevada	X	
06-17	8-Jul	Hwy 174	676442	4333220	Placer	X	
06-18	8-Jul	Stevens Trail	677496	4330463	Placer	X	
06-19	8-Jul	Pennyweight Tr	679397	4329862	Placer	X	
06-20	8-Jul	Yankee Jim Rd	680662	4329696	Placer	X	
06-21	8-Jul	Shirttail Cyn	686082	4329545	Placer	X	
06-22	8-Jul	Welcome Mine	684541	4325612	Placer	X	
06-23	8-Jul	Shirttail Cyn Ck	683200	4324214	Placer	X	
06-24	10-Jul	Coloma SP	682843	4297222	ElDorado		
06-25	10-Jul	Bucksbar Rd	699700	4280710	ElDorado		
06-26	10-Jul	Sly Park	711815	4289612	ElDorado		
06-27	10-Jul	Chili Bar	689248	4292973	ElDorado	X	
06-28	10-Jul	Traverse Ck	690920	4299778	ElDorado		
06-29	24-Jul	Freeman Ln	667137	4340501	Nevada		
06-30	24-Jul	Bear R / Hwy 49	664131	4319849	NevPlac	X	
06-31	24-Jul	Wolf Cr / Hwy 49	665414	4325424	Nevada		
06-32	24-Jul	Lotus	681200	4295460	ElDorado	X	

Table 3. Plants recorded on Vegetation Transects. California 2006.

Hosts *(including Associated / Nursery Hosts)*	
White fir	*Abies concolor*
Big leaf maple	*Acer macrophyllum*
Other maples	*Acer* spp. *
Madrone	*Arbutus menziesii*
Manzanita	*Arctostaphylos* spp.*
California hazel	*Corylus cornuta*
Coffeeberry	*Frangula californica*
Oregon ash	*Fraxinus latifolia*
Tanoak	*Lithocarpus densiflorus*
Oleander	*Nerium oleander*
Douglas-fir	*Psuedotsuga menziesii*
Black oak	*Quercus kelloggii*
Canyon live oak	*Quercus chrysolepis*
Rose	*Rosa* spp.*
Yew	*Taxus brevifolia*
Nutmeg	*Torreya californica*
Poison oak	*Toxicodendron diversilobum*
Bay laurel	*Umbellularia californica*
California buckeye	*Aesculus californica*
Toyon	*Heteromeles arbutifolia*

* *Although not identified to species, these genera contain host species within the survey area.*

Other Plants	
Chamise	*Adenostoma fasciculatum*
White Alder	*Alnus* spp.
Tree-of-heaven	*Ailanthus altissima*
Coyote bush	*Baccharis pilularis*
Chestnut	*Castanea sp.*
Redbud	*Cercis occidentalis*
Mountain misery	*Chamaebatia foliolosa*
Ceanothus	*Ceanothus* spp.
Cotoneaster	*Cotoneaster spp.*
Dogwood	*Cornus* spp.
Eucalyptus	*Eucalyptus spp.*
Fig	*Ficcus* spp.
Black Walnut	*Juglans nigra*
Ponderosa pine	*Pinus ponderosa*
Sugar pine	*Pinus lambertiana*
Grey pine	*Pinus sabiniana*
Cherry, plum	*Prunus spp.*
Incense-cedar	*Libocedrus decurrens*
Osage Orange	*Maclura pomifera*
Apple	*Malus sylvestris*
Mulberry	*Morus* spp.
Olive	*Olea europaea*
Poplar	*Populus* spp.
Pear	*Pyrus* spp.
Blue oak	*Quercus douglasii*
Valley oak	*Quercus lobata*
Interior live oak	*Quercus wislizeni*
Ribes	*Ribes* spp.
Locust	*Robinia* spp.
Blackberry	*Rubus* spp.
Willow	*Salix* spp.
Elderberry	*Sambucus* spp.
Giant Sequoia	*Sequoiadendron gianteum*
Snowberry	*Symphoricarpos* spp.
Periwinkle	*Vinca* spp.
Grape	*Vitis* spp.
Broom	different genera

Attachment

UC DAVIS-RIZZO LAB STREAM BAIT METHODS

Stream 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 stream, 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 stream 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 stream flow and away from edge/bank
- Flag rope with contact info
- Clean soil/mud off boots used for accessing stream (rubber boots work great)- use 95% Ethanol or 10% bleach water; optional if not infested stream course

Collection:

- Remove leaves from water and place in separate sample collection bags
- Rinse bag and leaves in stream if dirt and detritus on leaves/bag/rope
- Take water temperature of stream at pick up- leave thermometer in water +2min (this helps evaluate how long to leave baits in streams)
- 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.