California 2005 National *Phytophthora ramorum* Wildland Survey
California Department of Forestry and Fire Protection

Submitted by Donald R. Owen
September 2005

Summary

As part of the 2005 National *Phytophthora ramorum* wildland survey, detection surveys were conducted in eastern Butte and Yuba 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 35 vegetation transects were surveyed and 11 streams baited. Roughly 170 miles of roadside vegetation was scanned while driving through areas identified as moderate to high risk for sudden oak death. Eight vegetation samples were collected for lab diagnosis. *P. ramorum* was not detected by any of the survey methods. The only confirmed *Phytophthora* infection was from symptomatic bay leaves collected near Pulga, Butte County, which yielded *P. psuedosyringae*. This *Phytophthora* was also recovered during last year's survey from symptomatic bay leaves collected in San Luis Obispo County.

SUDDEN OAK DEATH / *P. ramorum* SURVEY

The quarantined pest *P. ramorum* is not established in the Sierra Nevada of California, although hosts and putatively suitable habitat occur there. Risk analyses identified eastern Butte and Yuba Counties as that portion of the Sierra Nevada having the most suitable habitat for establishment of *P. ramorum*. 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) Conduct a stream-baiting pilot project to recover *Phytophthora spp.* from the principal streams draining these habitats, and 3) conduct follow-up surveys if *P. ramorum* is recovered.

The project was planned and coordinated by CDF Entomologist Don Owen. Surveys were conducted by Owen and retired CDF Pathologist David Adams from late April to early August 2005. Cooperators included Ross Meentemeyer, formerly of Sonoma State University, who provided 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 provided the protocol, baits, and conducted the diagnostics for stream samples. Surveys were conducted on private land, portions of the Plumas and Tahoe National Forests, and the Oroville State Recreation Area. Dave Frazer of the Yuba River

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

Procedures

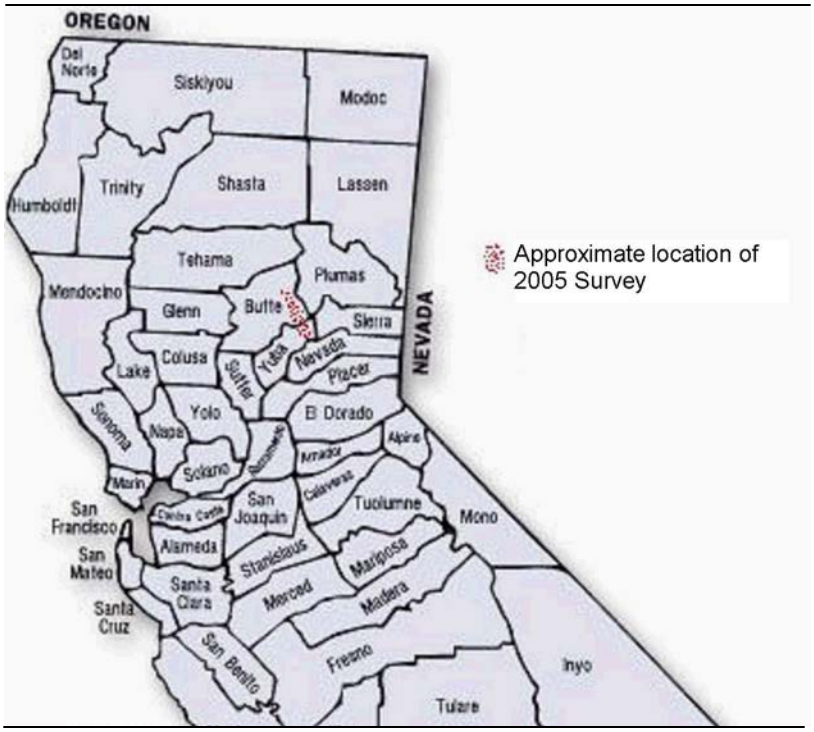
The Geographic Information Center, Sonoma State University, provided 1:100,000 scale maps delineating areas of moderate to high risk for the establishment of *P. ramorum* in the northern Sierra Nevada. These were overlaid with standard 1:100,000 USGS maps to identify access roads and streams that traverse 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 orthophoto maps for field use. A reconnaissance was made to determine accessibility and a total of 35 transect locations and 11 streams were chosen for the survey.

Streams were sampled using the protocol of the UC Davis – Rizzo Lab (Attachment 1), 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). Baits for the first sample were placed-out April 27 to May 4 and collected May 18-26; baits for the second sample were placed-out May 18-26 and collected June 13-15. Six of the streams were accessed by road and five were accessed by boat – 2 on Lake Oroville and 3 on New Bullard's Bar Reservoir. Samples were processed at UC Davis under the direction of Shannon Murphy 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/05/methods/survey_methods.doc). 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, which amounted to approximately 170 miles of survey through moderate to high-risk habitats. Samples for lab diagnosis were collected whenever symptoms of *P. ramorum* were encountered, including stem cankers and leaf spots. Each sample was divided in half and shipped to two separate labs - half to the Garbelotto Lab for PCR diagnostics and the other half to the Rizzo Lab for culturing. When leaf symptoms were encountered, an effort was made to collect a total of 20 symptomatic leaves per host species. 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 were reported every 2 weeks to USDA Forest Service, Forest Health Monitoring, WO (compiled as Attachment 2).



Results and Discussion

Eleven streams were initially baited, but flooding in mid-May resulted in some baits being lost or unrecoverable until water levels subsided. Mill Creek (Yuba Co.) became choked with large woody debris to such an extent that it was deemed too hazardous to sample and was removed from the survey. Ultimately, a total of 34 sets of baits were collected from 10 streams (Table 1). All of these streams drain either into the Feather or Yuba Rivers. No *Phytophthora* spp. were recovered from any of the baits.

Thirty-five transects were walked and visually scanned for hosts and symptoms of *P. ramorum* infection (Table 2). Fifteen hosts of *P. ramorum* were recorded (Table 3). The most common host was Douglas-fir, found on every transect. Tanoak was found on every transect but one. Madrone and big leaf maple were recorded on more than half the transects. The most common non-host was incense cedar, found on every transect. California bay laurel was only found on four transects, all within the Feather River drainage. We know from the combination of vegetation transects and stream samples

that bay laurel occurs somewhere within every major branch of the Feather River drainage – the West Branch and North, Middle, and South Forks. The largest numbers of bay were sheltered within steep drainages, of which Flea Valley and Mill Creeks in Butte County are good examples. It is suspected that most bay laurel within the survey area occur in inaccessible locations. Bay was not found on any of the areas surveyed within the Yuba River drainage.

Table 1. **Location of Stream Sites and Sampling dates.** California 2005.

	UTM		1st Sample Period		2nd Sample Period	
Stream Sites	**E**	**N**	**date out**	**date in**	**date out**	**date in**
Mill Creek (Butte Co)	633772	4407177	**4/29/2005**	5/26/2005	5/26/2005	6/14/2005
Bridger Cr	664071	4369303	**5/2/2005**	5/23/2005	5/23/2005	6/13/2005
Indian Cr	657583	4371431	**5/2/2005**	5/23/2005	5/23/2005	6/13/2005
Little Oregon Cr	657064	4365908	**4/27/2005**	5/182005	5/182005	6/14/2005
Middle Fork Yuba R	664909	4361990	**4/28/2005**	high water	**4/28/2005**	6/13/2005
Sucker Run	645871	4379591	**4/27/2005**	5/18/2005	5/18/2005	6/14/2005
Bryant Ravine	648764	4386193	**4/27/2005**	5/18/2005	5/18/2005	6/14/2005
French Cr	637643	4395310	**5/4/2005**	lost	5/26/2005	6/15/2005
Chino Cr	635221	4397795	**5/4/2005**	5/26/2005	5/26/2005	6/15/2005
Flea Valley Cr	632495	4407257	**4/29/2005**	5/19/2005	5/19/2005	6/14/2005
Mill Creek (Yuba Co)	660977	4368406	**5/2/2005**	lost	site discarded	

Table 2. Locations and dates of Vegetation Transects

			UTM					UTM	
ID	**Date**	**Location**	**E**	**N**	**ID**	**Date**	**Location**	**E**	**N**
DA 1	4-May	Concow	628892	4405134	V 18	28-Jul	Mosquito Cr	641129	4395705
DA 2	4-May	Forbestown	647053	4376637	V 19	28-Jul	Ram Cr	642621	4398096
DA 3	3-May	Challenge CO	650347	4373619	V 20	28-Jul	Jack Cr	638684	4397541
DA 4	2-May	N Challenge	652892	4374203	V 21	28-Jul	Upper Fr Cr	640663	4401090
DA 5	4-May	NE Challenge	654564	4373685	V 22	28-Jul	Peavine Cr	642204	4399300
DA 6	3-May	E Challenge	655207	4372444	V 23	24-Aug	U Galen Cr	640600	4393600
DA 7	2-May	Marysville Rd	665080	4367033	V 24	24-Aug	U Berry Cr	641477	4391500
DA 8	2-May	Old Toll Rd	664649	4365227	V 25	24-Aug	U Martin Cr	639992	4391887
DA 9	2-May	Marysville Rd	664138	4366159	V 26	24-Aug	U Martin Cr 2	639600	4391650
DA 10	28-Apr	Bridger Cr	663564	4371577	V 27	25-Aug	Soper Wh	647457	4372995
V 11	28-Apr	Baker Rd	660618	4369802	V 28	25-Aug	Soper Wh 2	646142	4373390
V 12	28-Apr	Mc Lain Rd	662055	4371962	V 29	25-Aug	Greenville	656120	4368162
V 13	late Apr	Flea Valley	632870	4407046	V 30	25-Aug	Fountain H Rd	655136	4367958
V 14	late Apr	Mill CalTrans	635284	4405604	V 31	31-Aug	Indian C 1	655550	4372455
V 15	26-Jul	Last Chance	635616	4400379	V 32	31-Aug	Indain C 2	656298	4371866
V 16	26-Jul	22N85	636138	4400844	V 33	31-Aug	(Middle Cr)	656650	4372465
V 17	26-Jul	Chino Cr	636775	4399406	V 34	31-Aug	Slapjack Cr	657308	4373229
					V 35	31-Aug	Empire C(upr)	658867	4374438

Table 3. Plants recorded on Vegetation Transects

Hosts	
Big leaf maple	*Acer macrophyllum*
Madrone	*Arbutus menziesii*
Manzanita	*Arctostaphylos* spp.*
Spice bush	*Calycanthus occidentalis*
California hazel	*Corylus cornuta*
Tanoak	*Lithocarpus densiflorus*
Honeysuckle	*Lonicera* spp.*
Douglas-fir	*Psuedotsuga menziesii*
Black oak	*Quercus kelloggii*
Canyon live oak	*Quercus chrysolepis*
Western Azalea	*Rhododendron occidentale*
Rose	*Rosa* spp.*
Yew	*Taxus brevifolia*
Poison oak	*Toxicodendron diversilobum*
Bay laurel	*Umbellularia californica*

* *because these were not identified to species, it is unknown if they are in fact hosts*

Other Plants	
White fir	*Abies concolor*
Mountain maple	*Acer glabrum*
Alder	*Alnus* spp.
Incense-cedar	*Libocedrus decurrens*
Ponderosa pine	*Pinus ponderosa*
Sugar pine	*Pinus lambertiana*
Ceanothus	*Ceanothus* spp.
Dogwood	*Cornus* spp.
Elderberry	*Sambucus* spp.
Ribes	*Ribes* spp.
Blackberry	*Rubus* spp.
Willow	*Salix* spp.
Grape	*Vitis* spp.
Broom	different genera

Despite the large number of hosts encountered, no symptoms of sudden oak death were found on any transects. Symptomatic host tissue was, however, collected at eight locations noted while driving between transects. Two samples were taken of bark cankers on dying tanoak (Indian and Mill Creek drainages, Yuba Co.), one was of twig dieback on tanoak (Mill Creek drainage, Yuba Co.), and the remainder where all leaf spots on bay (near Pulga, Butte Co.). None tested positive for *P. ramorum*. Two of the leaf samples were positive for another *Phytophthora* sp. -- *ilicis*-like (by PCR) or *P. psuedosyringae* (by culturing). The site in the Indian Creek drainage (UTM Coordinates 0656637 E 4371470 N) was initially sampled in April and involved several dying trees. It was returned to in August with the intention of collecting additional samples, but the trees had dried and deteriorated to the point that no cankers could be distinguished. This site will be revisited in the spring of 2006.

General Comments

The area surveyed contains an abundance of tanoak and other hosts of *P. ramorum.* Mean annual precipitation ranges from approximately 50-90 inches, ranking it among the wettest areas within the Sierra Nevada. Deep, relatively inaccessible canyons divide the terrain. Surveys were conducted between elevations of approximately 1,000-4,000 feet, the extremes of which represent canyon bottoms and adjacent ridges. Most stream samples were taken from streams with steep gradients and fast current.

Because of host abundance and climate, this area has some of the highest suitability for the establishment of *P. ramorum* within California's interior. Access, however, makes

the area difficult to monitor. The driving survey and vegetation transects covered the highest risk areas traversed by roads. Still, this was a small fraction of the total area that is rated as moderate to high risk. Stream baiting, in theory, samples a much larger area than could be covered by roadside survey. Finding access to the best stream sites, was, however, a challenging and time-consuming effort. Assuming that stream baiting effectively sampled all upstream habitats, then roughly ¾ of high risk habitats were sampled by this method. Stream baiting is, however, an experimental survey method of unknown efficacy.

If *P. ramorum* becomes established in this area, tanoak will likely be one of the first hosts to be seriously affected. Tanoak is found throughout the area and is highly susceptible to infection and disease caused by *P. ramorum*. Detection and sampling of dying tanoaks, particularly when groups of trees are involved, should thus be a prime objective of surveys for *P. ramorum* in this area. It is possible that bay laurel will have an important role in the establishment of *P. ramorum*. If this species is important or even essential to establishment, then some of the most inaccessible locations will have the most suitable habitat. Aerial survey is the best method for detecting dying tanoaks in such remote locations. Stream baiting potentially could detect the earliest infestations of *P. ramorum*, but this is unproven. Roadside surveys and transects are a good method for assessing basic host and access information, but provide the least coverage of at-risk habitats.

END OF REPORT
Attachments Follow

Attachment 1.

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.

Attachment 2. Reports to Forest Health Monitoring.

Report 1. 5-17-05
5. # of General Forest Locations Sampled:10
6. #of General Forest Samples Submitted:0
13. # of other samples submitted: 2
14. # of other samples PCR negative: pending
15 # of other samples PCR positive: pending

Report 2. 7-1-05
5. # of General Forest Locations Sampled: 10
6. #of General Forest Samples Submitted: 0
9. # of Aquatic Locations Sampled: 19 (this represents 2 locations for each of 9 streams and 1 location for 1 stream)
10. # of Aquatic Samples Submitted: 34 (this represents 2 sampling periods)
11. # of Aquatic Samples PCR Negative for Pr (of total submitted): pending
12. # of Aquatic Samples PCR Positive for Pr (of total submitted): pending
13. # of other samples submitted: 8
14. # of other samples PCR negative: pending *
15 # of other samples PCR positive: pending
* one sample was cultured and IDed as *Phytophthora psuedosyringae.*

Report 3. 7-14-05
5. # of General Forest Locations Sampled: 14
6. #of General Forest Samples Submitted: 0
9. # of Aquatic Locations Sampled: 19 (this represents 2 locations for each of 9 streams and 1 location for 1 stream)
10. # of Aquatic Samples Submitted: 34 (this represents 2 sampling periods)
11. # of Aquatic Samples PCR Negative for Pr (of total submitted): 34 **
12. # of Aquatic Samples PCR Positive for Pr (of total submitted): 0
13. # of other samples submitted: 8
14. # of other samples PCR negative: 8 *
15 # of other samples PCR positive: 0
* one sample was cultured and IDed as *Phytophthora psuedosyringae.* PCR tests indicated the same.
** Aquatic samples were submitted to UC Davis for determination. The protocol involves visual inspection and culturing. PCR testing would only be done if the researchers determined there was a need for it.

Report 4. 7-28-05
5. # of General Forest Locations Sampled: 22
6. #of General Forest Samples Submitted: 0
9. # of Aquatic Locations Sampled: 19
10. # of Aquatic Samples Submitted: 34
11. # of Aquatic Samples PCR Negative for Pr (of total submitted): 34
12. # of Aquatic Samples PCR Positive for Pr (of total submitted): 0
13. # of other samples submitted: 8
14. # of other samples PCR negative: 8
15 # of other samples PCR positive: 0

Report 5. 8-12-05
No change from above

Report 6. 8-29-05
5. # of General Forest Locations Sampled: 30
6. #of General Forest Samples Submitted: 0
9. # of Aquatic Locations Sampled: 19
10. # of Aquatic Samples Submitted: 34
11. # of Aquatic Samples PCR Negative for Pr (of total submitted): 34
12. # of Aquatic Samples PCR Positive for Pr (of total submitted): 0
13. # of other samples submitted: 8
14. # of other samples PCR negative: 8
15 # of other samples PCR positive: 0

Report 7 (FINAL). 9-2-05
5. # of General Forest Locations Sampled: 35
6. #of General Forest Samples Submitted: 0
9. # of Aquatic Locations Sampled: 19
10. # of Aquatic Samples Submitted: 34
11. # of Aquatic Samples PCR Negative for Pr (of total submitted): 34 **
12. # of Aquatic Samples PCR Positive for Pr (of total submitted): 0
13. # of other samples submitted: 8
14. # of other samples PCR negative: 8 *
15 # of other samples PCR positive: 0

* one sample was cultured and IDed as *Phytophthora psuedosyringae.* PCR tests indicated the same.

** Aquatic samples were submitted to UC Davis for determination. The protocol involves visual inspection and culturing. PCR testing would only be done if the researchers determined there was a need for it. No PCR testing was done on these samples.