

Monitoring *Phytophthora ramorum* Distribution in Streams within Coastal California Watersheds

S.K. Murphy¹, C. Lee², Y. Valachovic², J. Bienapfl¹, H.A. Patterson¹, K.F. Falk¹, and D.M. Rizzo¹

¹ Department of Plant Pathology, University of California, Davis, CA 95616, skmurphy@ucdavis.edu
² UC Cooperative Extension Humboldt and Del Norte Counties, 5630 S. Broadway, Eureka, CA 95503

ABSTRACT

Thirty-six locations were established in winter-spring 2004 to monitor for the presence of *Phytophthora ramorum* (Pr) in perennial watercourses throughout coastal northern California. The areas of focus include Alameda, Contra Costa, Del Norte, Humboldt, and Mendocino counties. These counties have limited Pr detection but are high-risk areas for Pr infestation. Two sites in Sonoma County are included as a baseline for successful recovery of Pr.

Rhododendron leaves are placed in mesh bags and secured in watercourses for 7-21 day intervals year-round to bait for *Phytophthora* species. Recovered symptomatic leaves are plated on *Phytophthora*-selective media.

Pr was recovered at all sites with a priori knowledge of Pr forest infestation. We recovered Pr at three sites downstream of known forest infestations. One site is along the South Fork Eel River, approximately 8km downstream of known infestation near Redway. Additionally, Pr was recovered at two sites without prior known forest infestation in Briones East Bay Regional Park.

Stream monitoring provides a useful method of early detection for Pr infestation. Future work will include additional monitoring at the southern extent of the known Pr range. In addition, we will address research questions related to spread and survival of this pathogen in watercourses.



Figure 2. Methods demonstration



Figure 1. Map of California stream monitoring sites.
 ★ Sites with *P. ramorum* recovered.



Figure 3. Symptoms on Rhododendron leaves infected by *P. ramorum* zoospores.

METHODS

- Clean, disease-free Rhododendron leaves are placed in 1mm sterilized fiberglass mesh bags at stream sites year-round every six weeks.
- 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 stream temperatures and longer intervals in cold conditions. Interval time adjusted year-round.
- Leaves are collected and placed in separate sample collection bags.
- Leaves are surface sterilized in 95% Ethanol for 30 seconds, rinsed with DI water, and air-dried for 1-2 hours.
- 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.
- Plates are incubated at 18°C for one month and checked microscopically twice weekly for growth of *Phytophthora* species.
- Any *Phytophthora*-like organisms are transferred and further examined for identification.

RESULTS

Site	Code(s) on Figure 1	Stream	County	Distance to Pram ¹	+ <i>P. ramorum</i> isolation				- no <i>P. ramorum</i> isolation				site not sampled	other species ²
					FEB	MAR	APR	MAY	JUN/JUL	AUG/SEP	OCT/NOV	DEC		
Redwood EBRP	RR	Redwood	Alameda	adjacent	+	+								unk Psp
Briones EBRP	BR1, BR2	Along and Beer	Contra	adjacent	+	+								unk Psp
Fairfield Osborn Preserve	FOP	Copeland	Sonoma	adjacent	+	+							+	unk Psp
Sonoma State University	SSU	Copeland	Sonoma	upstream 7km east	+	+								unk Psp
Mallard Redwoods State Reserve	MRW	Mill	Mendo	adjacent	-	+	+	-	-	-	-	-	-	Pgon
Boonville Property	RNCH	Rancheria	Mendo	adjacent				+	-	-	-	-	-	Pgon
Peachland Road Property-intstd	PCH1	Indian Creek	Mendo	adjacent				+	+	-	-	-	+	Pgon
Peachland Road Property-unintstd	PCH4	Indian Creek	Mendo	upstream 3km east				+	-	-	-	-	-	Pgon
Henry Woods SP	HND	Navarro R.	Mendo	east				-	-	-	-	-	-	Pgon
Brooktrails Township	DHC,WCK	Dutch Henry, Willis	Mendo	-				-	-	-	-	-	-	Pgon
UC Angelo Coast Reserve	ANG1, ANG2, ANG3	Elder, S. Fork Eel, & Fox	Mendo	-				-	-	-	-	-	-	Pps, Pgon
Benbow	EBE	S. Fork East Branch Eel	Humboldt	-				-	-	-	-	-	-	
Tooby Park, Gabeville	TOOB	S. Fork Eel	Humboldt	adjacent				-	-	-	-	-	-	
west of Redway	RWC	Redwood (S)	Humboldt	adjacent				+	+	-	-	-	-	Pgon
north of Redway	RED	S. Fork Eel	Humboldt	upstream 8km south				-	+	-	-	-	-	
Humboldt Redwoods SP	BUL	Bull	Humboldt	south				-	-	-	-	-	-	
Grizzly Creek Redwoods SP	GRZ	Grizzly	Humboldt	-				-	-	-	-	-	-	
Hay 299	LND	Lindsay	Humboldt	-				-	-	-	-	-	-	Pgon
Redwood NP	RNP	Redwood (N)	Humboldt	-				-	-	-	-	-	-	Pgon
Prairie Creek Redwoods SP	PRC	Prairie	Humboldt	-				-	-	-	-	-	-	Pgon
Hoopa Reservation	TSH, CAMP, SUPP, SOC, PNE, BUL2, MLC2	7 creeks	Humboldt	-				-	-	-	-	-	-	Pgon
Jedediah Smith SP	MLC1	Mill	Di Nte	-				-	-	-	-	-	-	
Six Rivers NF	RCK	Rock	Di Nte	-				-	-	-	-	-	-	Pgon
Six Rivers NF	HSC	Hardscrabble	Di Nte	-				-	-	-	-	-	-	Pgon
Six Rivers NF	PCK	Peacock	Di Nte	-				-	-	-	-	-	-	Pgon
public land near fish hatchery	RWD	Rowdy	Di Nte	-				-	-	-	-	-	-	Pgon

Table 1. 2004 Detection Results

¹ location and approximate distance to nearest known *P. ramorum* presence (from forest plant isolation) as a potential inoculum source of stream; adjacent-*P. ramorum* isolated from forest adjacent to stream site(s); upstream-*P. ramorum* isolated upstream from stream site(s) and approximate distance and direction is given

² Pgon= *P. gonapodyides*; Pps=*P. pseudosyringae*; unk Psp=sequence identified as unknown *Phytophthora* species

FUTURE WORK

- Monitor additional sites throughout northern California, including the Mattole River watershed and the Yurok Indian tribal lands.
- Monitor additional sites in coastal central California (southern range of *P. ramorum*), including Monterey and San Luis Obispo counties.
- Address research questions related to spread, survival, and quantification of *P. ramorum* in stream courses.

REFERENCES

E.J. Fichtner, S.C. Lynch, and D.M. Rizzo. Detection and Distribution of *Phytophthora ramorum* in Soils. Sudden Oak Death Science Symposium, January 18-21, 2005.

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