Stream Monitoring for Detection of *Phytophthora ramorum* in Oregon[1]

W. Sutton,[2] E.M. Hansen,[2] P. Reeser,[2] and A. Kanaskie[3]

Abstract

Stream monitoring using leaf baits for early detection of *P. ramorum* is an important part of the Oregon sudden oak death program. About 50 streams in and near the Oregon quarantine area in the southwest corner of the state are currently monitored. Rhododendron and tanoak leaf baits in mesh bags are exchanged every two weeks throughout the year. Leaves are sent to Corvallis for isolation and PCR diagnosis using the Winton and Hansen ITS primers. *P. ramorum* is regularly recovered from streams draining infested sites 5 years after eradication treatment. Because there has been a very good correspondence between culture results and PCR results from these known infested streams, we have changed to PCR only diagnosis on streams outside the known diseased area. If a PCR positive result is obtained, these baits and subsequent baits from that stream are cultured as well as assayed by PCR. In three watersheds *Phytophthora ramorum* was first detected using stream baiting. Follow up intensive ground surveys located infected tanoaks or other host plants. *Phytophthora ramorum* has been cultured from one additional stream but a source of inoculum has not yet been found.

Key words: *Phytophthora ramorum*, sudden oak death, PCR, stream monitoring.

Methods and Results

Rhododendron and tanoak leaf baits in mesh bags are floated in streams and exchanged every two weeks throughout the year. Leaves are sent to Oregon State University (OSU) for diagnostic testing. When stream monitoring first began in Oregon all bait samples were cultured using CARP+BH, a selective medium and PCR assay using the Winton and Hansen multiplex protocol. Over time confidence grew in the PCR assay so that currently initial testing of streams baits uses PCR only. If a PCR positive result is obtained from a stream not known to be infected, these baits and subsequent baits from that stream are culture and PCR assayed.

Culture vs PCR

Four years of data were analyzed comparing the two diagnostic methods (table 1). With PCR assay there are only two outcomes, positive and negative. PCR reliably detects *P. ramorum* in baits, even when it cannot be isolated in pure culture. When culturing however, there is a third outcome, another *Phytophthora* species.

---

[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

[2] Oregon State University, Botany and Plant Pathology, Corvallis, OR 97331. Corresponding author: Everett Hansen, hansene@science.oregonstate.edu.

[3] Oregon Department of Forestry, Salem, OR 97310.

Phytophthora species are abundant in forest streams, and may mask or prevent the growth of *P. ramorum*. While the system is not perfect, 89 percent of the time the outcomes agree, three percent are mismatched and eight percent are suspected to be culture suppression due to other *Phytophthora* or *Pythium* species, or *P. ramorum* no longer being viable.

Table 1—Correspondence between culture and PCR results for detection of *P. ramorum* in 1,148 stream bait samples collected from 2003 to 2006

Diagnosis		
Culture	*PCR*	*Percent*
P. ramorum	Positive	6
No Ph sp	Negative	25
P. ramorum	Negative	1
No Ph sp	Positive	2
Other Ph sp	Positive	8
Other Ph sp	negative	58

Seasonality

Phytophthora ramorum is detected in streams by culturing and diagnostic PCR of leaf baits in all seasons of the year (table 2).

Eradication Sites

Phytophthora ramorum is recovered regularly from streams draining infested sites 5 years after eradication treatment (table 3). Detection rate has declined from 70 percent of sample intervals in 2003 to 39 percent of intervals in 2006.

Table 2—Number and percentage of monthly intervals in which *P. ramorum* was detected by culture or PCR in five streams draining eradication sites in a four year period (2003-2006)

	Winter		Spring		Summer		Fall	
Culture	8/31	26%	10/34	29%	9/35	26%	15/31	48%
PCR	18/52	35%	37/60	62%	41/60	68%	29/50	58%

Table 3—Frequency of detection of *P. ramorum* in 5 streams draining SOD eradication sites

	2003		2006	
Stream	# sample intervals	*P. ramorum* positive intervals	# sample intervals	*P. ramorum* positive intervals
WA1	17	71%	19	53%
WA4	24	50%	22	32%
WA6	15	33%	23	13%
WA9	23	87%	24	46%
WA12	24	96%	24	54%
Total	103	70%	112	39%

Early Detection Tool

Tentative belief that stream monitoring could be used as an early detection tool for *P. ramorum* has been proven well-founded. *Phytophthora ramorum* was detected using stream baiting in several watersheds before any infected plants were found. Three streams illustrate the utility of stream baiting for early detection of *P. ramorum* in tanoak forests .

Stream WA46 drains tanoak forest, flowing into the North Fork Chetco river from the east. Baiting began in March 2004, and the first PCR positive results were obtained in June 2004. Repeated ground searches revealed nothing, until November 2005 when three infected tanoaks were found (table 4). The site received eradication treatments that winter.

Stream WA65 drains an old-growth redwood-tanoak-Douglas-fir stand on state and federal land. It was first baited for *P. ramorum* in May 2004, and was first culture and PCR positive in October 2005. Despite earlier searches, infected plants were not located until April 2006, and the site received eradication treatments that year (table 5).

WA 31 is 2 km north of the quarantine area. It has been baited since March 2004. It was first PCR positive in August 2004, but was then negative until August 2005, when *P. ramorum* was cultured from a leaf floating in the stream. The next five sample times were PCR positive (*table 6*). Repeated ground surveys had failed to find the source of inoculum at the time of this meeting. On March 6, 2007 the Oregon Department of Forestry field crew located an infected tanoak 152.4 m (500 ft) up stream from this stream bait site.

Table 4—Monthly diagnostic results for stream WA46

	Mar04	Apr04	May04	Jun04	Jul04	Aug04	Sep04	Oct04	Nov04	Dec04
WA46	neg	neg	POS	POS	POS	neg	0	neg	neg	neg

Jan05	Feb05	Mar05	Apr05	May05	Jun05	Jul05	Aug05	Sep05	Oct05	Nov05	Dec05
neg	neg	neg	neg	neg	neg	neg	POS	neg	neg	neg	neg

Jan06	Feb06	Mar06	Apr06	May06	Jun06	Jul06	Aug06	Sep06	Oct06	Nov06	Dec06
neg	neg	neg	neg	0	POS	POS	neg	neg	POS	neg	neg

Table 5—Monthly diagnostic results for stream WA65

				May04	Jun04	Jul04	Aug04	Sep04	Oct04	Nov04	Dec04
WA65				neg	neg	neg	neg	0	0	neg	neg

Jan05	Feb05	Mar05	Apr05	May05	Jun05	Jul05	Aug05	Sep05	Oct05	Nov05	Dec05
neg	neg	neg	neg	neg	neg	neg	neg	neg	POS	neg	neg

Jan06	Feb06	Mar06	Apr06	May06	Jun06	Jul06	Aug06	Sep06	Oct06	Nov06	Dec06
neg	neg	neg	neg	POS	neg	neg	POS	neg	POS	neg	neg

Table 6—Monthly diagnostic results for stream WA31

	Mar04	Apr04	May04	Jun04	Jul04	Aug04	Sep04	Oct04	Nov04	Dec04
WA31	neg	neg	neg	neg	neg	POS	0	neg	neg	neg

Jan05	Feb05	Mar05	Apr05	May05	Jun05	Jul05	Aug05	Sep05	Oct05	Nov05	Dec05
neg	neg	neg	neg	neg	neg	neg	POS	POS	POS	POS	neg

Jan06	Feb06	Mar06	Apr06	May06	Jun06	Jul06	Aug06	Sep06	Oct06	Nov06	Dec06
neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

Literature Cited

Winton, L. M.; Hansen, E.M. 2001. Molecular diagnosis of *Phytophthora lateralis* in trees, water, and foliage baits using multiplex polymerase chain reaction. Forest Pathology. 31: 275–283.