Chapter 8. *Phytophthora ramorum* Early Detection Surveys for Forests

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Introduction

Diseases caused by *Phytophthora ramorum* Werres, De Cock, & Man in't Veld including ramorum canker (also known as sudden oak death), ramorum blight, and ramorum dieback were first detected in U.S. forest landscapes in Marin County, CA, in the mid-1990s. The range in the United States has expanded since then, but in 2007 it was still limited to 14 central coastal California counties and a small area in Curry County, OR (U.S. Department of Agriculture, Animal and Plant Health Inspection Service 2007). Despite the presently limited disease distribution in forests and measures to limit artificial movement of the pathogen by State and Federal regulatory agencies through quarantine and restrictions on the movement of plant materials, other areas are at risk. This is especially true for oak-dominated forest ecosystems in the Eastern United States. In these areas, susceptibility of many closely related native Eastern United States trees and shrubs have been demonstrated in greenhouse inoculation trials (Tooley and Kyde 2007) and via natural infection in Europe (Brasier and others 2004). Climate parameters over much of the area are at least seasonally similar to those prevailing in *P. ramorum* endemic areas on the West coast, and brisk trade in many susceptible woody ornamental hosts grown there make accidental introduction an ever-present danger.

The risk of establishment of the pathogen outside the regulated areas and the potential for severe ecosystem damage prompted Federal and State forest management agencies in seven Eastern States to join in pilot tests of early detection terrestrial vegetation survey methods for forests. These surveys began in 2003, were expanded to more States, and continued annually through 2006 when aquatic survey methods were pilot tested concurrent with the terrestrial vegetation surveys in 11 States. Results of terrestrial vegetation surveys (Oak and others 2008a) and the 2006 pilot stream survey (Oak and others 2008b) have been presented. The purpose of this chapter is to present the combined results of the national *P. ramorum* early detection survey for U.S. forests using both terrestrial vegetation (2003 through 2006) (Oak and others 2008a) and stream baiting (2006 and 2007) survey protocols.

The occurrence and distribution of *Phytophthora* species in waterways in various environmental settings worldwide has been studied using filtration and plant tissue baits from a wide variety of plant species (Klotz and others 1959, McIntosh 1966, Robertson 1975, Thompson and Allen 1974, von Broembsen 1984). Recently, monitoring of *P. ramorum* in forest streams of California and Oregon has been shown to be effective using pear fruits and foliage of tanoak (*Lithocarpus densiflorus*)

and rhododendron (*Rhododendron* spp.) as bait (Hansen and Sutton 2006, Tjosvold and others 2006). The success of these methods for detecting *P. ramorum* in water, even before host symptoms are visible in aerial surveys, has resulted in early detection and treatment of previously unknown infested sites (Hansen and Sutton 2006, Murphy and others 2006). Given that previously unknown infection centers have been detected up to 8 km downstream from the inoculum source, monitoring forest streams for *P. ramorum* in high-risk regions should afford an opportunity to survey larger land areas with greater efficiency and at lower cost than is possible with current terrestrial vegetation survey techniques.

The 2006 pilot survey was highly successful in detecting multiple *Phytophthora* species in surveyed waterways (Oak and others 2008b). *Phytophthora* species were detected in over 80 percent of the bait sets diagnosed from 90 watersheds surveyed in 11 States. *P. ramorum* was detected in all streams known by previous sampling to be positive and for the first time in one Washington stream not previously known to harbor the pathogen. Because of these results, stream baiting protocols replaced terrestrial vegetation surveys in the 2007 national *P. ramorum* early detection survey for U.S. forests.

Methods

Terrestrial Vegetation Surveys—Terrestrial survey methods have been described in detail by Oak and others (2006). Briefly, the survey is guided by projected risk based on abundance and distribution of putatively susceptible hosts, climatic match with endemic West coast areas, and potential pathways of introduction via woody ornamental nursery stock. Up to 30 survey locations per cooperating State are used in two types of field settings—wooded nursery perimeter and general forest area. Four 100-m transects of variable width (dependent upon host type density) are used to survey each location. Symptomatic host or associated host plant (HAP) species (U.S. Department of Agriculture, Animal and Plant Health Inspection Service 2007) are targeted where available, while species belonging to HAP genera are targeted outside the endemic States of California and Oregon. The presence of *P. ramorum* in or on host tissues is verified by nested or realtime Polymerase Chain Reaction (PCR) according to U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA–APHIS) approved protocols (DeVries and Levy 2006, Levy and Mavrodieva 2004). Replicates of each sample are tested at both local and regional laboratories in order to

minimize the possibility of unnecessary action based on false positive or uncertain results from a single diagnostic procedure.

Stream Surveys—Protocols closely followed those established for the 2006 pilot survey (Oak and others 2008b). Each stream is baited with four detached but otherwise unwounded *Rhododendron* spp. leaves contained in a mesh bag made of plastic window screen. Bags are tethered to streambanks and floated in the current for 1 to 2 weeks (exposure period depended on symptom development). After retrieval, bait leaves are wrapped in a paper towel moistened with streamwater, sealed in a plastic bag, and placed on ice for transport to diagnostic laboratories.

Bait leaves are washed under running tap water to remove silt and organic debris and blotted dry. Symptomatic leaf pieces are then excised without surface disinfestation. The only significant departure from the 2006 protocol is the mandatory use of isolation on selective medium in local diagnostic laboratories, while regional laboratories use nested or realtime PCR.

Results

Terrestrial Vegetation Surveys—*P. ramorum* forest surveys were conducted in at least 1 year between 2003 and 2006 in a total of 39 States (table 8.1). Of the 25 States that confirmed *P. ramorum* in woody ornamental nurseries during this period (California Oak Mortality Task Force 2009) only Arizona, Colorado, and New Mexico did not conduct surveys. Projected risk in these States is low. Nursery perimeters represented 62 percent of the 3,570 locations, reflecting our judgment that these settings were most likely to show symptoms the earliest, given the large shipments of potentially infected woody ornamental nursery stock in 2004 and subsequent, smaller shipments in each year thereafter. Local and regional laboratories diagnosed a combined 12,699 symptomatic tissue samples, and only 2 were found positive for *P. ramorum*. Both of these were *Quercus agrifolia* bark samples collected in San Francisco County, CA.

Symptomatic tissues from 44 identified genera were submitted for molecular diagnostics. Samples from species of *Acer* and *Lonicera* were the most abundant (table 8.2), while samples

Table 8.1—Summary survey statistics 2003–06

Survey characteristic	Year				Overall
	2003	2004	2005	2006	
Cooperating States	7	36	39	36	39
Locations surveyed					
Nursery perimeter	44	881	682	607	2,214
General forest	128	304	487	437	1,356
Total	172	1,185	1,169	1,044	3,570
Samples diagnosed	1,092	4,263	3,328	4,016	12,699
Phytophthora ramorum positive diagnosis	0	2	0	0	2

Table 8.2—Frequency and rank of plant genera sampled and submitted for *Phytophthora ramorum* diagnosis. Only genera ranked among the top 10 in any survey year are shown

Sample genus	Overall		Year							
			2003		2004		2005		2006	
	No.	Rank	No.	Rank	No.	Rank	No.	Rank	No.	Rank
Acer	2,642	1	11	6	970	1	829	1	832	1
Lonicera	2,068	2	17	5	712	3	639	2	700	2
Unidentified	1,637	3	284	2	850	2	208	7	295	4
Kalmia	1,318	4	363	1	437	4	277	4	241	5
Quercus	1,204	5	175	4	295	5	416	3	318	3
Rhododendron	933	6	231	3	257	6	244	5	201	6
Vaccinium	655	7	0	–[a]	234	7	218	6	203	7
Viburnum	366	8	3	8	108	8	130	8	125	10
Rubus	237	9	0	–[a]	37	13	4	22	196	8
Prunus	214	10	0	–[a]	64	10	20	17	130	9
Hamamelis	213	11	0	–[a]	83	9	71	9	59	16
Aesculus	136	12	0	–[a]	43	11	46	10	47	17
Castanea	48	20	8	7	27	14	5	21	8	25
Total	12,699		1,092		4,263		3,328		4,016	

[a] Rank is undefined due to absence of samples.

from unidentified genera ranked third in abundance. Symptomatic leaves were by far the most common tissue type, accounting for 94 percent of all samples. Bark from stem hosts represented < 5 percent of samples.

Stream Surveys—Overall, in 2007, 152 streams were surveyed in 28 cooperating States for the requisite 5 baiting periods or more (fig. 8.1). The extra bait deployments resulted in a possible 871 bait sets (table 8.3). Some bait sets were partially or completely lost due to high storm flows, vandalism, or other causes. In the case of partial losses, diagnosis by isolation only was performed. Overall, < 4 percent of bait sets were lost.

Previous surveys showed that *P. ramorum* was present in 11 streams surveyed in California and Oregon. These served as positive checks. The pathogen was detected in baits from all of these check streams at least once in five baiting periods by either direct isolation, PCR, or both diagnostic methods. In addition, *P. ramorum* was detected in two streams that were not known to harbor the pathogen prior to the 2007 survey. One stream was in Washington and was different from the stream found positive in the 2006 pilot survey (Oak and others 2008b), while the other was in Mississippi. The watersheds drained by both streams contained one or more confirmed positive woody ornamental nurseries. *P. ramorum* negative diagnoses for other streams are supported by the fact that *Phytophthora* spp. were detected by isolation in over 80 percent of all bait sets diagnosed nationally, including recovery of multiple species in *P. ramorum* positive streams.

PCR was more sensitive than isolation for detecting *P. ramorum* in known positive streams (table 8.4). However, the need for replicate diagnostics was demonstrated by the fact that the pathogen was detected by isolation, but not PCR, in baits from the Washington stream, while the reverse was true for baits from the Mississippi stream. Further, single positive PCR results were obtained from baits deployed in several California and Oregon streams not known to harbor the pathogen. This also occurred in the 2006 pilot survey (Oak and others 2008b). The small number of these cases combined with the location far from known inoculum sources and the lack of repeat positives through time by either diagnostic method suggests the possibility of false positive PCR diagnosis.

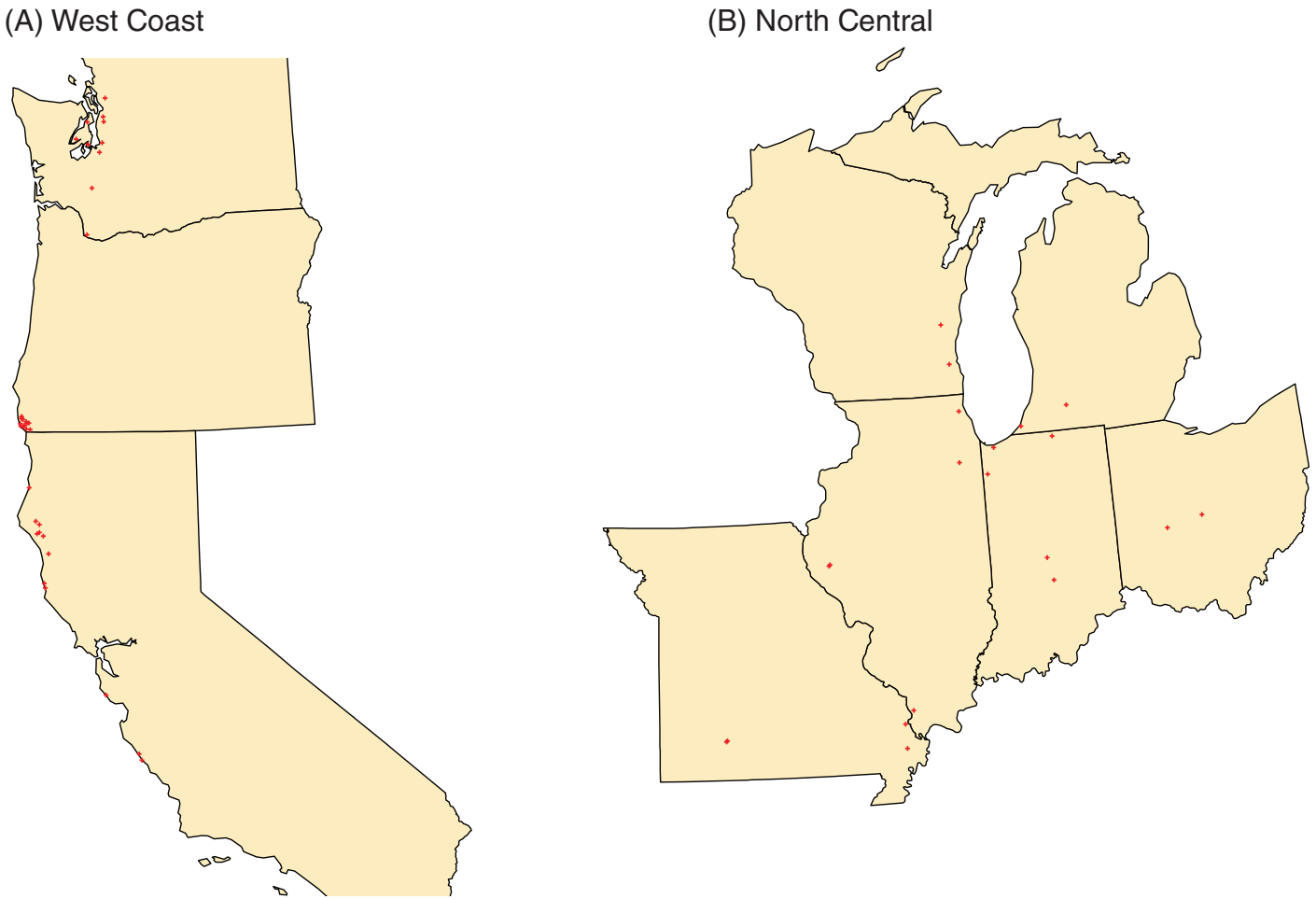

Figure 8.1—Locations of streams surveyed in the 2007 Phytophthora ramorum *early detection survey in forests. Note: Maps in this figure are not to scale in relation to one another.*

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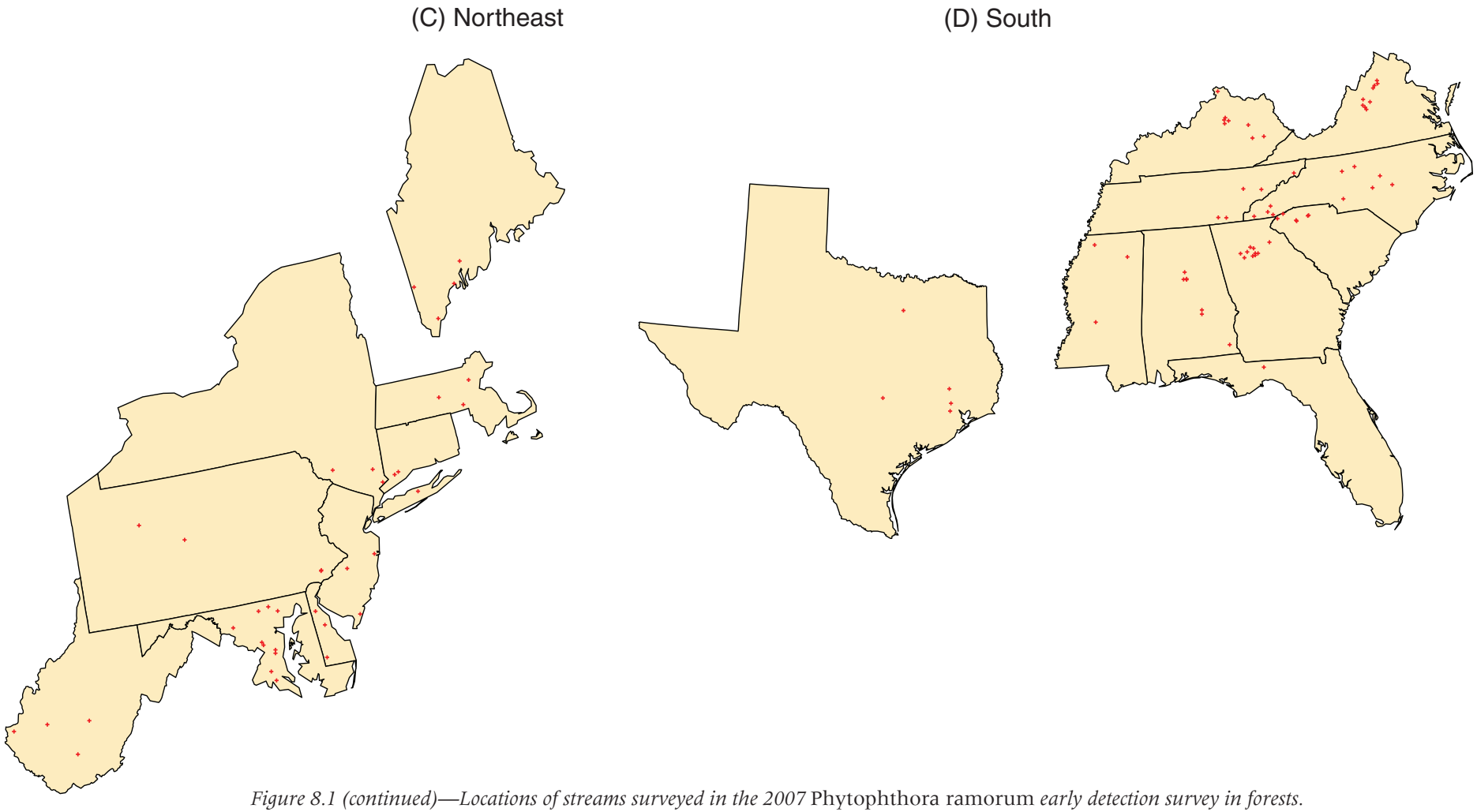

Figure 8.1 (continued)—Locations of streams surveyed in the 2007 Phytophthora ramorum *early detection survey in forests.*

Table 8.3—Summary stream baiting survey statistics, 2007

Survey characteristic	Region				Total
	West Coast	North Central	Northeast	South	
Cooperating States	3	6	9	10	28
Streams surveyed					
Phytophthora ramorum positive	12[a]	0	0	1	13
P. ramorum negative	22	18	36	63	139
Total	34	18	36	64	152
Bait sets diagnosed					
Isolation subtotal	259	90	180	317	846
P. ramorum positive	22	0	0	0	22
Other *Phytophthora* spp. positive	166	88	139	265	658
All *Phytophthora* spp. positive (percent)	188 (72.6)	88 (97.8)	139 (77.2)	265 (83.6)	680 (80.4)
PCR subtotal	257	88	181	305	831
P. ramorum positive	53	0	0	1	54

PCR = Polymerase Chain Reaction.
[a] *Phytophthora ramorum* was known to be present in 11 West coast streams prior to survey. These streams served as positive checks.

Table 8.4—Summary diagnostic statistics for *Phytophthora ramorum* positive streams, 2007

State	Streams	*Phytophthora ramorum* diagnosis					
		Isolation			Polymerase chain reaction		
		Attempts	Positive	Percent	Attempts	Positive	Percent
California	9	43	9	20.9	84	33	39.3
Oregon	2	26	12	46.2	26	19	73.1
Washington	1	9	1	11.1	9	0	0
Mississippi	1	5	0	0	5	1	20
All	13	83	22	26.5	124	53	42.7

Discussion

Terrestrial surveys conducted from 2003 through 2006 resulted in the addition of San Francisco County, CA, to the regulated area, consolidating the central coastal California distribution of the pathogen to include 14 counties. No other detections were made by sampling symptomatic plants from HAP genera in 4 years of terrestrial survey. However, in only 2 years of rhododendron leaf baiting (the first year being a limited pilot survey) the pathogen was detected in three streams in locations far removed from endemic areas. Two of these streams were in western Washington while the third was in Mississippi. The positive detections by stream baiting surveys exceeded those made by terrestrial vegetation surveys in half the time from 20 percent of the samples and 4 percent of the locations. This demonstrates the distinct advantage of stream baiting over terrestrial vegetation surveys for *P. ramorum* early detection.

While the pathogen was detected in streams outside regulated areas on the West coast, the source of inoculum could not be identified with certainty. Intensive surveys failed to detect infections in streamside plants. The proximity of confirmed positive woody ornamental nurseries to the positive streams suggests them as sources, but some detections were made after infected plants were removed and destroyed during the implementation of the USDA–APHIS confirmed nursery protocol. Possible inoculum sources include infected, but as yet undetected plants in the suspect nurseries, including crop as well as noncrop plants; soil-borne inoculum that was not eradicated during implementation of the confirmed nursery protocol; infected plants sold and planted in ornamental landscapes; and infected plants in or near the surveyed streams that escaped detection, perhaps even plants not yet known to be hosts. Monitoring of *P. ramorum*-positive streams and environs will continue until inoculum sources are identified and eradicated.

Overall, these results support the conclusion that *P. ramorum* is not widely established in U.S. forests, even in proximity to nurseries where the pathogen has been confirmed in infected woody ornamental stock located in high-risk areas where host type and climate are conducive to disease development. However, not all infected plants were intercepted at the nurseries, and it is certain that some are planted in residential and commercial landscapes in high-risk areas. Introductions from infested West coast nurseries have continued annually from 2003 through 2007. The length of latent periods between introduction, establishment in native vegetation, and detection are unknown. These facts dictate continued early detection efforts to maximize the efficacy of eradication of new outbreaks.

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Literature Cited

Brasier, C.M.; Denman, S.; Rose, J. [and others]. 2004. First report of ramorum bleeding canker on *Quercus falcata*, caused by *Phytophthora ramorum*. Plant Pathology. 53: 804.

California Oak Mortality Task Force. 2009. Nursery chronology. http://www.suddenoakdeath.org/html/chronology.html. [Date accessed: Unknown].

DeVries, R.M.; Levy, L. 2006. Quantitative multiplex real-time PCR (qPCR) for detection of *Phytophthora ramorum* using a TaqMan System on the Cepheid SmartCycler® and the ABI 7900/7000. USDA–APHIS plant health; pest information; *Phytophthora ramorum*; real-time PCR protocol. Rev. 4, April 3, 2006. 11 p. http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/WI-B-T-1-6Rev4.pdf. [Date accessed: Unknown].

Hansen, E.M; Sutton, W. 2006. Persistence of *Phytophthora ramorum* after eradication efforts in Oregon tanoak forests [Poster]. In: Brasier, Clive; Jung, Thomas; Oßwald, W., eds. Proceedings of the 3rd international IUFRO working party 07.02.09 meeting. Farnham, UK: Forest Research. P. 178.

Klotz, L.J.; Wong, P.P.; DeWolfe, T.A. 1959. Survey of irrigation water for the presence of *Phytophthora* spp. pathogenic to citrus. Plant Disease Reporter. 43: 830–832.

Levy, L.; Mavrodieva, V. 2004. PCR detection and dna isolation methods for use in the *Phytophthora ramorum* national program. USDA–APHIS. plant health; pest information; *Phytophthora ramorum*; nested PCR protocol. 11 p. http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/pcrprotocol4.pdf. [Date accessed: Unknown].

McIntosh, D.L. 1966. The occurrence of *Phytophthora* spp. in irrigation systems in British Columbia. Canadian Journal of Botany. 44: 1591–1596.

Murphy, S.K.; Lee, C.; Bienapfl, J. [and others]. 2006 Monitoring *Phytophthora ramorum* distribution in streams within coastal California watersheds [Poster]. In: Frankel, S.J.; Shea, P.J.; Haverty, M.I., tech. coords. Proceedings of the sudden oak death second science symposium: the state of our knowledge. Gen. Tech. Rep. PSW–GTR–196. Albany, CA: Forest Service, U.S. Department of Agriculture, Pacific Southwest Research Station: 531.

Oak, S.W.; Smith, W.D.; Tkacz, B.M. 2006. *Phytophthora ramorum* detection surveys for forests in the United States. In: Brasier, Clive; Jung, Thomas; Oßwald, W., eds. Proceedings of the 3rd international IUFRO working party 07.02.09 meeting. Farnham, UK: Forest Research: 28–30.

Oak, S.W.; Elledge, A.H.; Yockey, E.K. [and others] 2008a. *Phytophthora ramorum* early detection surveys for forests in the United States, 2003–2006. In: Frankel, Susan J.; Kliejunas, John T.; Palmieri, Katherine M., tech. coords. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW–GTR–214. Albany, CA: Forest Service, U.S. Department of Agriculture, Pacific Southwest Research Station: 413–416.

Oak, S.W.; Hwang, J.; Jeffers, S.N.; Tkacz, B.M. 2008b. 2006 pilot survey for *Phytophthora ramorum* in forest streams in the USA. In: Frankel, Susan J.; Kliejunas, John T.; Palmieri, Katherine M., tech. coords. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW–GTR–214. Albany, CA: Forest Service, U.S. Department of Agriculture, Pacific Southwest Research Station: 59–64.

Robertson, G.I. 1975. A paper disc technique for the recovery of *Pythium* spp. from soil or water. New Zealand Journal of Agricultural Research. 18: 409–410.

Thompson, S.V.; Allen, R.M. 1974. Occurrence of *Phytophthora* species and other potential plant pathogens in recycled irrigation water. Plant Disease Reporter. 57: 391–393.

Tjosvold, S.A.; Chambers, D.L.; Koike, S.; Fichtner, E. 2006. Epidemiology of *Phytophthora ramorum* infecting rhododendrons under simulated nursery conditions. In: Frankel, Susan J.; Shea, Patrick J.; Haverty, Michael I., tech. coords. Proceedings of the sudden oak death second science symposium: the state of our knowledge. Gen. Tech. Rep. PSW–GTR–196. Albany, CA: Forest Service, U.S. Department of Agriculture, Pacific Southwest Research Station: 459–461.

Tooley, P.W.; Kyde, K.L. 2007. Susceptibility of some eastern forest species to *Phytophthora ramorum*. Plant Disease. 91: 435–438.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service. 2007. Plant health; pest information; *Phytophthora ramorum*; http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/index.shtml. [Date accessed: Unknown].

von Broembsen, S.L. 1984. Distribution of *Phytophthora cinnamomi* in rivers of the Southwestern Cape Province. Phytophylactica. 16: 227–229.