Phytophthora ramorum Early Detection Surveys for Forests in the United States, 2003–2006[1]

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Abstract

Risk-based early detection surveys in U.S. forests were conducted between 2003 and 2006 using 100 m vegetation transects. Thirty-nine states surveyed 3,570 locations in states with endemic *Phytophthora ramorum*; states where the pathogen had been confirmed only in woody ornamental nurseries; and states that had received potentially infected stock but did not confirm the pathogen. A total of 12,699 samples from 44 host and associated host genera were collected and diagnosed for the pathogen using nested or real-time PCR. *Phytophthora ramorum* positive diagnoses were obtained for two samples from San Francisco County, California confirming that the pathogen is not yet widely established outside the regulated areas on the west coast.

Key words: *Phytophthora ramorum*, sudden oak death, survey, risk rating.

Introduction

Diseases caused by *Phytophthora ramorum* in U.S. forest landscapes were first detected in Marin County, CA in the mid-1990s. The endemic range in the U.S. has expanded since then, but in 2007 is still limited to14 central coastal California counties and a small area in Curry County, Oregon (USDA-APHIS. 2007). Despite limited disease distribution in forests, the vulnerability of oak-dominated forest ecosystems in the eastern U.S. is suggested by the demonstration of susceptibility of many closely related native eastern U.S. trees and shrubs in greenhouse inoculation trials (Tooley and others 2007) and via natural infection in Europe (Brasier and others 2004), and by brisk trade in many of these susceptible woody ornamental hosts. This report updates statistics already presented for early detection surveys conducted in 2003 and 2004 (Oak and others 2006).

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Methods

The risk of establishment of the pathogen outside the regulated areas prompted federal and state forest management agencies in seven eastern U.S. states to join in pilot tests of early detection survey methods for forests in 2003. Climatic variables, abundance and distribution of putatively susceptible hosts, and potential pathways of *P. ramorum* introduction on woody ornamental nursery stock were combined in a risk map (Oak and others2006) used to guide sampling intensity. Cooperators were asked to survey 30 locations within their state with an emphasis on high risk areas. Field settings were of two types- wooded nursery perimeter and general forest area. Four-100 m transects were used to survey each location for symptomatic plants. Transect width was not fixed, but rather was determined by host type density. The minimum width was approximately 3 m in extremely dense rhododendron thickets, while the maximum width in sparse oak woodland could be about 30 m. West coast forest host and associated host species do not grow in the pilot survey states, and there was uncertainty as to the full host range of the pathogen. Therefore, target hosts were eastern species in genera of known hosts or associated hosts. Replicates of symptomatic bark, leaf and twig tissues of *Quercus*, *Kalmia*, and *Rhododendron* species were targeted for collection when present, and sent to local and regional laboratories for nested PCR diagnosis of *P. ramorum* according to United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) approved protocol (Levy and others 2004). Replicate testing was conducted as mutual confirmation of suspect positives to minimize the possibility of unnecessary action based on uncertain results.

The survey was implemented operationally in 2004 with mostly minor modifications following the accidental introduction of the pathogen to nurseries and landscapes throughout the country via infected woody ornamentals from a southern California nursery. The most significant modifications were an increase in the number of cooperating states, and expansion of the target host list (11 genera, plus any species displaying suspect aerial *Phytophthora* disease symptoms). Real-time PCR was added as a diagnostic technique after it was certified and approved by USDA-APHIS (DeVries and others2006). Methods remained mostly unchanged in 2005 and 2006.

Results and Discussion

Phytophthora ramorum forest surveys were conducted in at least one year between 2003 and 2006 in a total of 39 states (table 1). Of the 24 states that confirmed *P. ramorum* in ornamental nurseries during this period, only Arizona, Colorado and New Mexico did not conduct surveys, but projected risk in these states is low. Nursery perimeters represented 62 percent of the 3,570 locations reflecting the judgment that these settings were most likely to show symptoms 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 12,699 symptomatic tissue samples and only two were found positive for *P. ramorum*. Both of these were *Q. agrifolia* bark samples collected in San Francisco County, California.

Symptomatic tissues from 44 identified genera were submitted for molecular diagnostics. Samples from *Acer* species were the most abundant (table 2) while 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 less than 5 percent of samples.

Table 1—Summary survey statistics 2003-2006

	Year				
	2003	**2004**	**2005**	**2006**	**Overall**
Cooperating states	7	36	39	36	39
Locations surveyed					
Nursery perimeter	44	881	682	607	2214
General forest	128	304	487	437	1356
Samples Diagnosed	1092	4263	3328	4016	12699
P. ramorum Positive Diagnosis	0	2	0	0	2

Table 2—Frequency and rank of plant genera sampled and submitted for *P. 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	2642	1	11	6	970	1	829	1	832	1
Lonicera	2068	2	17	5	712	3	639	2	700	2
Unidentified	1637	3	284	2	850	2	208	7	295	4
Kalmia	1318	4	363	1	437	4	277	4	241	5
Quercus	1204	5	175	4	295	5	416	3	318	3
Rhododendron	933	6	231	3	257	6	244	5	201	6
Vaccinium	655	7	0		234	7	218	6	203	7
Viburnum	366	8	3	8	108	8	130	8	125	10
Rubus	237	9	0		37	13	4	22	196	8
Prunus	214	10	0		64	10	20	17	130	9
Hamamelis	213	11	0		83	9	71	9	59	16
Aesculus	136	12	0		43	11	46	10	47	17
Castanea	48	20	8	7	27	14	5	21	8	25
Grand Total	**12699**		**1092**		**4263**		**3328**		**4016**	

These surveys resulted in the addition of San Francisco County, California to the regulated area, consolidating the central coastal California distribution of the pathogen to include 14 counties. However, no new detections outside of this area were made. These results further support the conclusion that *P. ramorum* is not widely established in U.S. forests, even in proximity to nurseries where it has been confirmed in infected woody ornamental stock 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. Continued annual introductions from 2003 through 2007 have occurred. 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. Recent advances in sampling of waterways for *P. ramorum* will result in changes in national early detection survey protocols. Transect surveys will be replaced with water sampling using rhododendron leaf bait in 2007.

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