

# Detection of *Phytophthora ramorum* at Retail Nurseries in the Southeastern United States<sup>1</sup>

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## Abstract

Many nursery plants are known to be hosts of *Phytophthora ramorum* or to be associated with this pathogen. These plants can be infected or merely infested by *P. ramorum* and with or without symptoms. The pathogen has been detected most frequently on container-grown nursery plants, and occasionally has been found in the container mix around these plants. Because of this close association with container-grown nursery plants, *P. ramorum* can be disseminated within a nursery, among local nurseries, and between nurseries in different states on infected plants or infested materials. Pathogen propagules can be moved readily from nurseries or landscapes to natural ecosystems in soil and water. Therefore, a major concern is that *P. ramorum* will be introduced into nurseries in the eastern United States (U.S.) and then will become established in forests and other natural areas where environmental conditions are favorable for pathogen survival and reproduction and susceptible plants are present. Consequently, we have been cooperating with the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS) to monitor for *P. ramorum* in soil and water at retail nurseries in the southeastern U.S. when infected plants have been found by routine surveys.

To detect *P. ramorum* in field soil and container mix, a representative composite sample of at least 1 liter was obtained by collecting and combining multiple subsamples (100 to 200 ml each). Subsamples of field soil (composed of soil, gravel, peat fines, and other substrates) were collected to a depth of 10 to 20 cm around and under container-grown plants known or suspected to harbor the pathogen and along the drainage path of runoff water leaving an area of suspect plants. Subsamples of container mix were collected from all depths in pots containing plants with or without typical symptoms of *Phytophthora* foliage blight—usually with one subsample taken from each plant in a block. In the laboratory, each soil sample was mixed thoroughly and tested for *Phytophthora* spp. with a baiting bioassay. Three subsamples were removed from each composite sample and flooded with distilled water. Small pieces of camellia and rhododendron leaves (5 × 5 mm) were floated on the water, and baited soils were held at 20 °C for 3 days. Baits were removed, rinsed in distilled water, blotted dry, and embedded in PARPH-V8 selective medium. Isolation plates were held at 20 °C for up to 4 weeks and were examined regularly for colonies of *P. ramorum*.

To detect *P. ramorum* in water, samples (1 or 2 liters in volume) were collected in 100 ml aliquots from irrigation runoff water in ponds, streams, retention basins, drainage ditches, and puddles on nursery property. Samples were assayed by filtration within 6 hours of collection. For each sample, eight or nine aliquots (50 to 200 ml each) were passed through membrane filters with 3 or 5 µm pores. Filters were inverted onto PARPH-V8 selective medium, and

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plates were held at approximately 20 °C in the dark for 2 to 3 days. Filters then were removed and colonies of *Phytophthora* spp. were counted; representative colonies were subcultured and saved for identification. Plates were examined for colonies of *P. ramorum* for up to 21 days.

**Table 1—Occurrence of *P. ramorum* at five garden centers and nurseries in three states from 2006 through early 2009: on plants, in irrigation runoff water, and in soil associated with diseased plants**

Site	Date		<i>P. ramorum</i> + plants <sup>a</sup>	Irrigation runoff <sup>b</sup>		"Soil" <sup>c</sup>	
	Year	Month		Pond	Ditch	Pots	Field
FL: Retail garden center	2006	Feb	camellia	-	+		+
		Mar		-	-		-
	2007	Mar	camellia	+	-		+
		Apr		-	-		+
		Dec		-		-	-
2008	Feb	viburnum, loropetalum	-	-	+	+	
FL: Production nursery	2008	Feb			+		+
	2009	Mar			-		-
MS: Retail garden center	2008	Feb	magnolia		+		+
		Apr 01	magnolia		+		
		Apr 29	??		-		
	2009	Jan			+		-
		May			-	-	-
SC: Retail garden center	2008	Jun	kalmia				
		Jul	azalea, pieris		-	+	+
	2009	May	pieris			-	+
SC: Retail nursery	2008	Nov	rhododendron				
		Dec	rhododendron		-	+	+
	2009	Jan	kalmia, pieris		-	-	+
		Feb				-	
		May	rhododendron			-	+

<sup>a</sup>Plants infected by *P. ramorum* usually were diagnosed based on samples sent to state or federal laboratories.

<sup>b</sup>Irrigation runoff water was sampled in a holding pond at one Florida location and in drainage ditches.

<sup>c</sup>Soil samples were collected from field soil under diseased container-grown plants and from the container mix in the pots containing diseased plants.

Five nurseries in three states have been sampled repeatedly between 2006 and early 2009 (table 1). We also have detected *P. ramorum* at other retail nurseries and garden centers in Alabama, Georgia, and North Carolina. *P. ramorum* continues to occur on container-grown plants in retail nurseries in the southeastern U.S. The source of infection on these plants was not always clear; however, evidence suggests two scenarios: (1) plants already were infected before arriving at the nursery or garden center or (2) plants became infected after arriving at the nursery or garden center. Plants that were infected before arriving at nurseries and garden centers did not come from nurseries only in California, Oregon, and Washington, where *P. ramorum* is most common. This pathogen has escaped from diseased plants into field soil and has become established at some of these locations—where resident inoculum appears to have caused new infections on otherwise healthy plants. *P. ramorum* also is moving off-site in runoff water at some nurseries and garden centers. At several locations, runoff water was headed to forested ecosystems and local waterways. It is likely that infected or infested plants

have been sold and may have been planted in residential landscapes. Because *P. ramorum* has been found only where intensive surveys have been conducted, it is very likely that it is present at nurseries and garden centers in other southeastern states. Now it must be determined if *P. ramorum* has moved into the natural environment in the southeastern U.S. The sampling efforts at each of the five nurseries and garden centers listed in table 1 are summarized below.

**Florida.** From 2006 to 2008, a retail garden center in northern Florida was surveyed six times for *P. ramorum*. In February 2006, *P. ramorum* was found in runoff water in a retention basin and a connecting drainage ditch and in field soil. The ditch carried runoff water off-site to a wooded natural area. Infected camellias with sporulating lesions were present at the nursery; these plants had been received recently from an out-of-state nursery. *P. ramorum* was not detected in samples collected 1 month later. Infected camellias received from an out-of-state nursery again were present in early 2007 (prior to sampling in March) but were moved inadvertently to an off-site production nursery operated by the garden center. In samples collected in March 2007, *P. ramorum* was detected in field soil where diseased plants had been placed and in a pond where runoff water collected prior to leaving the garden center. The pathogen was detected again in a field soil sample collected 1 month later, but all samples collected in December 2007 were negative. In February 2008, viburnum and loropetalum plants at the garden center were found to be infected by *P. ramorum*. It also was detected in the field soil under and around these plants as well as in the container mix in the pots. It appeared that the inoculum initiating infections on these plants was being splashed up from the infested soil. During a visit to the production nursery where infected camellias from the garden center had been moved in 2007, *P. ramorum* was detected in field soil where the camellias had been located and in runoff water draining this location, which was moving away from the nursery into a forested area.

**Mississippi.** A retail garden center in Mississippi was surveyed five times, three times in 2008 and twice in 2009. Although thorough sampling in the garden center was not permitted until May 2009, a few soil samples were collected by state personnel and shipped to us in 2008 and early 2009. *P. ramorum* was detected in a field soil sample collected around the edge of an asphalt-paved pad where infected magnolia plants were located in February 2008, and it was repeatedly detected in runoff water leaving the nursery and heading for a local creek, which meandered through a forested area.

**South Carolina.** A retail garden center and a separate retail nursery in South Carolina were surveyed several times in 2008 and 2009. Infected kalmia plants were found at the garden center in June 2008. In July, *P. ramorum* was isolated from azalea and pieris plants, container mix in these pots, and field soil from around these plants, but it was not detected in water within and immediately outside the nursery. Infected pieris plants that were not present the previous summer were found in May 2009 during a follow-up survey, and the pathogen was recovered in a field soil sample from an adjacent block of plants. At the second location, the retail nursery was found to have diseased rhododendrons in November 2008, and *P. ramorum* was detected in soil samples collected in December. However, it was not detected in container mix samples collected from additional plants at this nursery in February 2009. *P. ramorum* was detected again in May 2009 on rhododendron plants that had been received recently from out-of-state nurseries. It was present in field soil under and around these plants but was not detected in the container mix from these pots. Most of the symptoms on the diseased plants were on lower branches, which suggested that the inoculum was resident in and splashing up from the field soil around these plants.