

# Comparison of *In Situ* and *In Vitro* Baiting Assays for *Phytophthora ramorum* Survey of Waterways in the Southeastern United States<sup>1</sup>

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## Abstract

*In situ* baiting with whole, intact leaves of *Rhododendron* spp. has been employed since 2006 by the National *Phytophthora ramorum* Early Detection survey of forests (national survey). Using this method, *P. ramorum* was detected for the first time in national survey waterways draining 12 infested ornamental crop nurseries in Alabama, Florida, Georgia, Mississippi, North Carolina, and Washington as well as many forest areas in California and Oregon. *In situ* baiting periods lasting 1 to 3 weeks allow sampling large volumes of water over time, but also can result in loss of bait leaves from storm surges and vandalism. *In situ* baiting also requires two site visits for a single bait set (once to deploy and once to retrieve) and sustained water flow. An *in vitro* assay without these limitations was evaluated in experimental applications, and it has been effective at recovering *P. ramorum*. Therefore, we used both the *in situ* and *in vitro* baiting assays simultaneously for the 2011 National Survey for 12 *P. ramorum*-infested waterways in five states in the southeastern United States to compare relative performance under field conditions.

*In situ* baiting was conducted according to the established national survey protocol, with three baiting periods during each of the spring and autumn seasons (six in all). Initiation of spring baiting varied with latitude and surveyor scheduling, ranging from February 14 to March 23, and was concluded by May 2. Fall baiting was conducted between September 21 and December 2. Eight collections of water samples were made for the *in vitro* assay—at the same time leaves were deployed or retrieved for *in situ* baiting. For the *in vitro* assay, two 800 ml water samples were collected in 100 ml aliquots, and each sample was placed in a 1 L Nalgene screw-top bottle. Each sample was baited immediately with 20 freshly cut leaf pieces and one whole, asymptomatic, non-wounded leaf of forest-grown *Rhododendron maximum* L. Bottles were capped, placed on their sides, and held for 3 days at 18 to 22 °C in the dark. Baits then were removed, rinsed in distilled water, and blotted dry. Leaf pieces were processed immediately for detection, while whole leaves were placed in moist chambers for up to 14 days to allow lesion development. Two detection methods were used for both assays—isolation on selective PARPH-V8 medium and nested or real-time PCR. Relative assay performance was determined by comparing *P. ramorum* detection results for sample sets collected at the same time.

There were 72 total cases for comparison of relative assay performance possible (12 sites x six baiting periods). However, site F1 was available for sampling only in the first spring baiting period, leaving 67 comparable cases. *Phytophthora ramorum* was recovered by one or both assays at least once during the year in 11 of 12 waterways surveyed (fig. 1), with more than double the detections occurring during spring (31) than autumn (15). *Phytophthora ramorum* was recovered by one or both assays in 32 of these cases (48 percent). Out of the 32 positive cases, the pathogen was recovered by both assays in 14 cases (44 percent), while each assay alone recovered the pathogen in nine cases (28 percent).

Pathogen recovery by each baiting assay differed considerably by season. During spring, there were 20 comparable cases in which *P. ramorum* was recovered by one or both assays. *In vitro* baiting recovered the pathogen in seven of these cases without corroboration by the *in situ* assay, while *in situ* baiting recovered the pathogen in two cases without *in vitro* corroboration. The pathogen was recovered by both assays in 11 additional cases. Relative performance of the two assays was exactly the inverse during autumn: two pathogen

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recoveries by *in vitro* baiting only, seven recoveries by *in situ* baiting only, and three recoveries by both methods.

Site	Spring Baiting Periods						Autumn Baiting Periods					
	1		2		3		4		5		6	
	IV	IS	IV	IS	IV	IS	IV	IS	IV	IS	IV	IS
A1												
A2												
A3												
A4												
A6												
M2												
N3												
N5												
G9												
G0												
G3												
F1												

Figure 1—*Phytophthora ramorum* recovery for 12 waterways in the southeastern United States by baiting season and assay method (IV = *in vitro*; IS = *in situ*). Shaded cells indicate *P. ramorum* positive; blackened cells indicate baits unavailable.

Despite seasonal differences, *in vitro* baiting was equal to *in situ* baiting for detection of *P. ramorum* in water during the 2011 baiting year, and demonstrated superiority in several instances. The detection of the pathogen by *in vitro* baiting was the first ever at site A1, despite 29 *in situ* baiting periods over 5 years. *In situ* baits were deployed, but lost due to flooding, during the first spring baiting period at site M2. However, *in vitro* samples could be safely collected and *P. ramorum* was recovered. Most importantly, *P. ramorum* would have escaped detection altogether at five sites had the *in vitro* assay not been used (A1, A2, A4, G0, and G3). There were no sites where *in situ* baiting demonstrated this advantage.

*In situ* baiting has proven effective for recovery of *P. ramorum* from water since 2006. Even though the *in vitro* assay samples a very small volume of water at only one point in time relative to *in situ* baiting, this did not prove to be disadvantageous for pathogen recovery in this survey. This fact suggests that waters draining infested areas in the eastern United States contain inoculum at detectable densities most of the time, at least during our spring baiting season. *In vitro* methods allow the sampling of intermittent waters, such as ephemeral drainages and puddled irrigation water in ornamental crop nurseries suspected of containing infested plants, as well as perennial streams in a variety of settings where *P. ramorum* has been introduced. Plans are in place to repeat this comparison survey at these sites in 2012, and expand it to include west coast forest and nursery sites. Changes to the national survey protocol will be considered if results prove repeatable.