

Detecting *Phytophthora ramorum* and Other Species of *Phytophthora* in Streams in Natural Ecosystems Using Baiting and Filtration Methods¹

Jaesoon Hwang,² Steven W. Oak,³ and Steven N. Jeffers⁴

Abstract

Phytophthora spp. occur widely in forest and other natural ecosystems. Because these straminipiles are well adapted to aquatic environments, monitoring strategically selected streams may reflect occurrence and distribution of *Phytophthora* spp. over the relatively large area drained by these streams. The mountain region of western North Carolina, in the southern Appalachian Mountains, was designated as a high risk area for sudden oak death, caused by *P. ramorum*, based on the occurrence of numerous native host plants, a relatively mild climate, and the prevalence of nursery businesses in this region that import plants or plant material from areas known to be infested. Therefore, five streams in three watersheds in Pisgah National Forest in western North Carolina were sampled monthly for *Phytophthora* spp. from April 2005 to March 2006 to determine if *P. ramorum* was present in the region, to determine the diversity of species of *Phytophthora* native to the region, and to compare baiting and filtration as detection methods. For baiting, either four wounded or four non-wounded leaves of *Rhododendron maximum* (a plant native to this region) were placed in a mesh bait bag made with nylon screen and PVC pipe (fig. 1). Wounded leaves were floated in a stream for 3 days while non-wounded leaves were exposed for 2 to 3 weeks. Water soaked lesions had developed on wounded leaves after 3 days in the water, and dark brown necrotic lesions were observed on non-wounded leaves exposed for 2 to 3 weeks. In the laboratory, five pieces of symptomatic leaf tissue were taken from each leaf, a total of 40 leaf pieces were embedded in PARPH-V8 selective medium to isolate *Phytophthora* spp. for each stream. For filtration, one liter of water was collected from each stream, and samples were filtered within 10 hours of collection. Nine 100-ml subsamples of water were vacuum-filtered (fig. 2) through two types of membrane filters (47-mm in diameter) with three pore sizes (Nuclepore with 1- and 3- μ m pores and Durapore with 5- μ m pores), and filters were inverted on PARPH-V8 medium to recover propagules of *Phytophthora* spp. trapped on the filters (fig. 3).

P. ramorum was not found in any of the streams in western North Carolina, but *Phytophthora* spp. were detected consistently from all five streams throughout the sampling period. To date, *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. gonapodyides*, *P. heveae*, *P. pseudosyringae*, and seven morphologically and genetically distinct groups of isolates have been identified from 1560 isolates collected. *P. gonapodyides* was most prevalent (1353 isolates) and was detected consistently in all months. *P. citricola*, *P. gonapodyides*, and *P. pseudosyringae* were distributed widely and recovered from all five streams. Isolation of *Phytophthora* spp. varied depending on month, location, and detection method. Diversity of *Phytophthora* spp. was greatest in July when 11 species and groups were recovered and least in February when only one species (*P. gonapodyides*) was recovered. *Phytophthora*

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

² Research Associate, 120 Long Hall, Dept. of ESPS, Clemson University, Clemson, SC 29634-0315.

³ Forest Pathologist, USDA-FS, Southern Region FHP, Asheville, NC 28802.

⁴ Associate Professor, 120 Long Hall, Dept. of ESPS, Clemson University, Clemson, SC 29634-0315.



Figure 1—Nylon mesh bag used to float wounded or non-wounded rhododendron leaves in forest streams. The PVC tube provides support and buoyancy.



Figure 2—A 100 ml sub-sample of stream water was transferred by pipette and then pulled through a membrane filter with the aid of a vacuum.

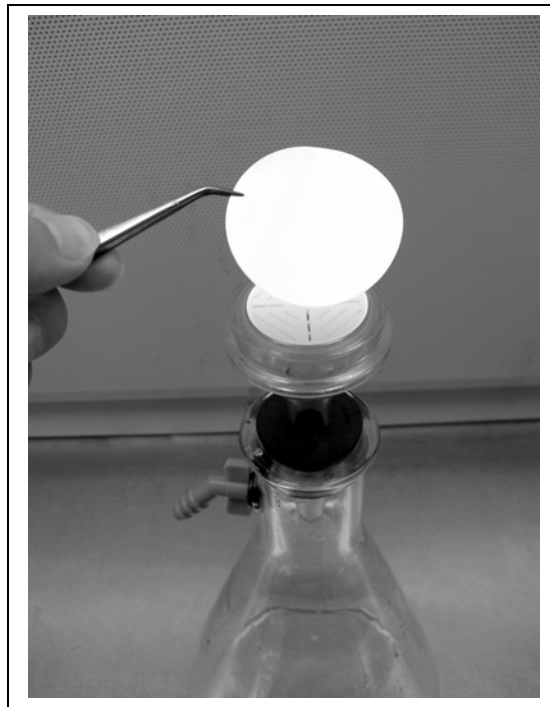


Figure 3—A 47 mm diameter membrane filter on which propagules of *Phytophthora* spp. have been trapped will be inverted onto PARPH-V8 selective medium to isolate the species present.

gonapodyides and *P. pseudosyringae* were the only two species detected from November to February in all streams. The greatest diversity in a single stream occurred in the South Mills river where 10 species and groups were detected over the sample period, and the least diversity was observed in Big Creek where four species and groups were found. Over the entire study period, 13 of the 14 species and groups were detected by filtration while only eight species and groups were isolated with each baiting method. Types or pore sizes of membrane filters did not affect detection of propagules of *Phytophthora* spp. Numbers of colonies recovered from Nucleopore 1- μ m, Nucleopore 3- μ m, and Durapore 5- μ m filters were 307, 331, and 264, respectively. Eight species and groups were trapped by Nucleopore 1- μ m and Durapore 5- μ m filters while nine species and groups were isolated with Nucleopore 3- μ m filters.

Filtration was validated as an effective method for detecting *P. ramorum* in streams in California where this pathogen previously had been found. In May 2005, three streams in Santa Cruz county were sampled and *P. ramorum* was detected in each one. In December 2005, *P. ramorum* was detected in four of eight streams across four counties (Marin, Monterey, Santa Cruz, and Sonoma). Densities of *P. ramorum* in waterways in Santa Cruz county were significantly lower in December than in May. From Lompico Creek, 36 (51 percent) of 70 isolates of *Phytophthora* spp. detected in May were *P. ramorum* whereas only two (4 percent) of 52 isolates of *Phytophthora* spp. detected in December were *P. ramorum*. Filtration was more effective and efficient than either baiting method for detection of diverse populations of *Phytophthora* species in forest streams. Filtration also provided quantitative data on inoculum density.

Key words: Diversity, forest streams, detection, membrane filter, *Rhododendron maximum*.

Acknowledgments

Authors wish to thank Marie Carras, Lynn Luszcz, Frank Martin, Patricia de Sa, Nina Shishkoff, Paul Tooley, Pedro Uribe, and Drew Zwart for technical assistance in isolation and species identification; Janice Alexander, David Chambers, Susan Frankel, and Steve Tjosvold for assistance with stream sampling in California; Jill Baker for assistance with stream sampling in North Carolina; North Carolina Arboretum and Pisgah Ranger District in Pisgah National Forest for providing access to study locations. This project was funded by United States Department of Agriculture - Agricultural Research Service, Foreign Disease-Weed Science Research Unit, USDA - Forest Service Pacific Southwest Research Station, and Armstrong Wood Products Inc.