Monitoring Occurrence and Distribution of *Phytophthora* Species in Forest Streams in North Carolina Using Baiting and Filtration Methods[1]

Jaesoon Hwang,[2] Steven W. Oak,[3] and Steven N. Jeffers[2]

Abstract

The recent epidemics of sudden oak death, caused by *Phytophthora ramorum*, in the coastal forests of California and southwest Oregon have drawn attention to other species of *Phytophthora* present in natural ecosystems that may threaten forest tree health. Since *Phytophthora* species are well adapted to aquatic environments, the species present in a strategically-selected stream may represent the occurrence and distribution of *Phytophthora* species over the relatively large land area drained by this stream. Based on this assumption, five streams in three watersheds in the Pisgah National Forest in western North Carolina were monitored monthly for *Phytophthora* species. from April 2005 to March 2006. Filtration and two baiting methods were used to detect propagules of *Phytophthora* species in stream water. A 1-liter water sample was collected from each stream and vacuum-filtered with Nuclepore and Durapore membrane filters. In addition, wounded or non-wounded leaves of *Rhododendron maximum* were used as baits and were exposed in a stream for 3 days or 2 to 3 weeks, respectively.

Phytophthora species were detected from all five streams throughout the 12-month monitoring period. *P. cambivora, P. cinnamomi, P. citricola, P. citrophthora, P. gonapodyides, P. heveae, P. pseudosyringae*, and seven morphologically and genetically distinct groups of isolates were identified from 1560 isolates collected. *P. gonapodyides* was the most prevalent (1353 isolates) and was detected consistently throughout the monitoring period. *P. citricola, P. gonapodyides*, and *P. pseudosyringae* were widely distributed and occurred in all five streams. Diversity of *Phytophthora* species was greatest in July, when 11 species/groups were detected and least in February when only one species was detected. *P. gonapodyides* and *P. pseudosyringae* were the only two species recovered over winter months (from November to February). Thirteen of the 14 species/groups were detected by filtration while only eight species/groups were isolated with each baiting method. Overall, filtration was superior to baiting for detecting *Phytophthora* species in forest streams: it provided quantitative data on propagule density; it was more efficient than either baiting method; and it was more effective at detecting greater species diversity.

Introduction

The recent epidemics of sudden oak death, caused by *Phytophthora ramorum*, in the coastal forests of California and southwest Oregon have drawn attention to other species of *Phytophthora* present in natural ecosystems that may threaten forest tree

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[2] Department of Entomology, Soils, and Plant Sciences; Clemson University; Clemson, SC 29634-0315, USA.
[3] USDA Forest Service, Southern Region FHP; Asheville, NC 28802, USA.
Corresponding author: jaesooh@clemson.edu.

health. Since species of *Phytophthora* are well adapted to aquatic environments, the species present in streams may represent the occurrence of *Phytophthora* species over most or all of the relatively large land area drained by them. Consequently, five streams were sampled monthly for 12 months to determine if streams could be used to monitor occurrence and diversity of *Phytophthora* spp. in a forest watershed.

Materials and Methods

Five streams in four distinct watersheds in the Pisgah National Forest in western North Carolina were sampled monthly from April 2005 to March 2006: Bent Creek, Big Creek, Fletcher Creek, Davidson River, and South Mills River. Filtration and two baiting methods were used to detect propagules of *Phytophthora* spp. in stream water. For filtering, a 1-liter sample of water was collected from each stream, and the samples were processed within 10 h of collection. Nine 100-ml sub-samples of water were vacuum-filtered through two types of membrane filters (47 mm in diameter) with three pore sizes: Nuclepore filters with 1- and 3-μm pores and Durapore filters with 5-μm pores. The filters were inverted onto PARPH-V8 selective medium in 100-mm-diameter disposable petri plates, and the plates were placed in the dark at 20°C for two to three days. Filters then were removed and colonies of *Phytophthora* spp. were counted. Representative colonies were subcultured and stored for later identification.

For baiting, either four wounded leaves or four non-wounded leaves of *Rhododendron maximum* were placed in a mesh bag rigged to float just below the water surface. Leaves were wounded by making cuts (5 to 10 mm in length) perpendicular to the leaf edge at approximately 5- to 7-mm intervals along the leaf edge (fig 1). Wounded leaves were exposed in a stream for three days while non-wounded leaves were exposed in the same stream for two to three weeks. To isolate *Phytophthora* spp. from wounded leaves, five rectangular pieces (approximately 5 to 7 mm on a side) with water-soaked lesions (fig 1) were cut from the edge of each leaf and embedded in PARPH-V8 medium. To isolate from non-wounded leaves, five pieces (approximately 5 mm × 5 mm) were removed from the margin area of water-soaked or necrotic lesions on each leaf and transferred to PARPH-V8 medium. Isolation plates were held at 20°C in the dark for 14 days and examined regularly for mycelia characteristic of *Phytophthora* spp. Representative colonies were subcultured and stored for later identification.

Figure 1—Water-soaked lesions on a wounded rhododendron leaf after 3 days of exposure in a forest stream.

Phytophthora species were identified based on morphological and molecular characters. Isolates grown on PARPH-V8 medium were examined for the presence of sporangia, chlamydospores, and oospores. In addition, agar plugs with actively growing hyphae were removed, flooded with 1.5 percent non-sterile soil extract solution, and placed under fluorescent lights at room temperature (22 to 24°C) for 24 h to induce sporangium production. For molecular characterization, each isolate was placed in pea broth medium at room temperature for five to seven days to produce a mycelium mat for DNA extraction. The ITS region of ribosome DNA and *cox* region of mitochondrion DNA were amplified using ITS4 and ITS6 or FM75 and FM77/83 primers sets. RFLP analysis was done with *Alu*I and *Msp*I restriction enzymes for ITS region DNA and with *Alu*I, *Rsa*I, and *Taq*I restriction enzymes for DNA from the *cox* region (fig 2).

Over the course of monitoring these streams, native plants of *Rhododendron maximum* and *Kalmia latifolia* with symptoms of tip dieback and leaf blight were observed in the riparian zone along all five streams. Shoots on some of these plants appeared to have been submerged in the streams during periods of high water flow. Foliage samples from symptomatic plants were collected, and tissue pieces from lesions were embedded in PARPH-V8 medium to isolate *Phytophthora* spp. as described above. In addition, composite soil samples were collected from the riparian zone of all five streams and assayed for *Phytophthora* spp. using a baiting bioassay. Approximately 1-liter of soil was collected along each stream. Samples were mixed thoroughly, and three replicate 100-ml aliquots of each soil sample were flooded with distilled water (200 ml of water per 100 ml of soil) and baited with leaf pieces of camellia and rhododendron. After three days at room temperature, leaf-piece baits were transferred to PARPH-V8 medium and plates were placed in the dark at 20°C for seven to ten days. Colonies of *Phytophthora* spp. were isolated and identified as described above.

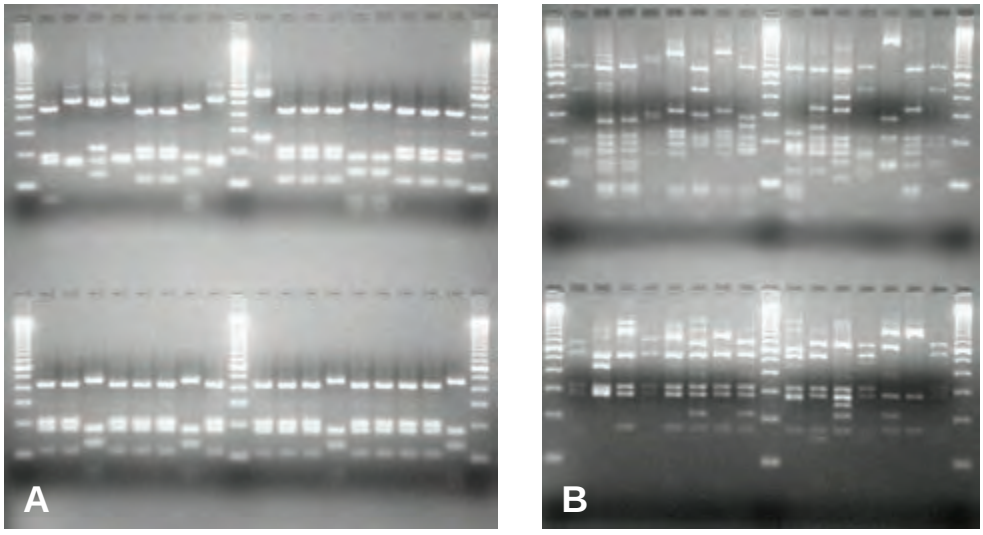

Figure 2—Identification of isolates of *Phytophthora* spp. by RFLP analyses: **A**, DNA from the ITS region of 34 isolates was amplified and digested with *Alu*I; **B**, DNA from the c*ox* region of 15 isolates was amplified and digested with *Alu*I (top) and *Rsa*I (bottom). Lanes (both top and bottom) 1, 10, and 20 in **A** and lanes 1, 10, and 18 in **B** contain a molecular marker.

Results and Discussion

Species of *Phytophthora* were detected in all five streams throughout the 12-month monitoring period. From 1,560 isolates collected, seven recognized species—*P. cambivora, P. cinnamomi, P. citricola, P. citrophthora, P. gonapodyides, P. heveae,* and *P. pseudosyringae*—and seven other morphologically and genetically distinct groups were identified. *P. gonapodyides* was most prevalent (1,353 isolates) and was detected consistently throughout the monitoring period. *P. citricola, P. gonapodyides,* and *P. pseudosyringae* were widely distributed and occurred in all five streams. The number and diversity of species of *Phytophthora* recovered varied depending on the month, location, and detection method. Diversity of *Phytophthora* spp. was greatest in July, when 11 species and groups were detected, and least in February—when only one species was detected. *P. gonapodyides* and *P. pseudosyringae* were the only two species recovered in winter months (November to February). The greatest diversity in a single stream occurred in the South Mills River where ten species and groups were detected over the 12-month sample period. Diversity was least in Big Creek where only four species and groups were found. Thirteen of the 14 species and groups were recovered by filtration while only eight species and groups were recovered with each baiting method. Overall, filtration was superior to either baiting method for detecting *Phytophthora* spp. in forest streams; it provided quantitative data on propagule density, recovered a greater diversity of species, and was more efficient because only one trip was needed to sample each stream. *P. citricola* and *P. heveae* were the only two species isolated from the samples of symptomatic plants collected along the five streams, and only *P. cinnamomi* and *P. heveae* were recovered from the riparian soil samples. In conclusion, *Phytophthora* spp. were abundant throughout the year in the five North Carolina forest streams we monitored, and there appeared to be a greater diversity of

species of *Phytophthora* in the streams than in the soils and plants along these streams—based on the methods employed in this study.

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