Variation in Density and Diversity of Species of *Phytophthora* in Two Forest Stream Networks[1]

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Abstract

Monitoring occurrence and distribution of *Phytophthora* species, including *Phytophthora ramorum*, in forest ecosystems can be achieved in several ways including sampling symptomatic plants, infested soils, and infested streams. Collecting plant and soil samples can be laborious and time consuming due to the distance surveyors need to travel. Not all forests are available for survey because of limited accessibility and stand density. Species of *Phytophthora* are well adapted to aquatic habitats and more diverse populations of *Phytophthora* spp. are found in forest streams than in nearby soils and symptomatic riparian plants. The current protocol for the National *P. ramorum* Early Detection Survey, conducted by the U.S. Department of Agriculture Forest Service (USDA FS), adopted sampling forest streams using baiting and filtration procedures. One assumption being made in these monitoring efforts is that species present in a stream network are representative of those present in the land area drained by that network. Therefore, careful selection of stream networks is essential to optimize the sampling effort with limited available resources, and strategic selection of sample sites within a stream network should maximize detection of the species of *Phtyophthora* present. A stream network in a natural ecosystem consists of a main stream, its tributaries, and a drainage point of the main stream. In this study, our hypothesis was that the occurrence and diversity of *Phytophthora* spp. at the drainage point of the main stream represents the overall population of *Phytophthora* spp. within the upstream network. If our hypothesis is true, a stream network could be surveyed effectively at the drainage point without additional sampling of the upstream tributaries.

Two stream networks located in the Pisgah National Forest in western North Carolina were selected to test our hypothesis. The Davidson River stream network (watershed size of 35.2 km^2) is composed of the Davidson River and nine individual tributaries that drain nine sub-watersheds (fig. 1). Seven tributaries, each in a separate sub-watershed, and the drainage point at the lower end of the Davidson River were sampled in September and October 2007. The Cathey's Creek stream network (watershed size of 29.6 km^2) consists of a main stream, Cathey's Creek, and eight tributaries—each draining a sub-watershed (fig. 2). The drainage point of Cathey's Creek and eight tributaries were sampled in June and October 2008. The drainage points and tributaries in each stream network were sampled within a 30-minute time period to minimize potential temporal variation. Later, a second sample was collected at the drainage point when water from the tributary farthest upstream was estimated to reach this point based on a real-time stream flow model. A 1 liter water sample was collected at each sample site; nine 100-ml aliquots were filtered through polycarbonate membrane filters with 3-µm pores, and filters were inverted onto PARPH-V8 selective medium. After 72 hours, filters were removed, and colonies of *Phytophthora* spp. were counted. A bait bag with four

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detached *Rhododendron maximum* leaves was deployed at each drainage point to compare recovery of species of *Phytophthora* by filtration and leaf baiting. Bait leaves were retrieved 2 to 3 weeks after deployment and leaf disks taken from the edges of lesions were embedded in PARPH-V8 medium. Representative isolates were subcultured and identified based on morphological and molecular characters.

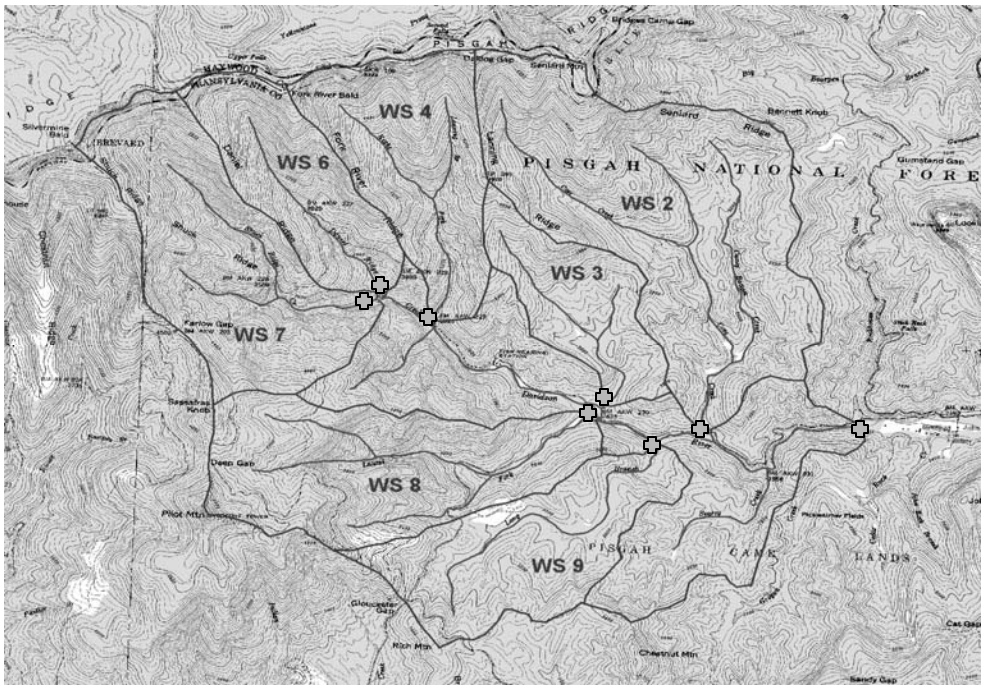

Figure 1—Sample sites (+), each in a distinct sub-watershed (WS), in the Davidson River stream network: the lower drainage point on Davidson River, Cove Creek (WS 2), Daniel Ridge Creek (WS 6), Laurel Fork (WS 8), Long Branch (WS 9), “No Name” Creek (WS 3), Right Fork (WS 4), and Shuck Ridge Creek (WS 7).

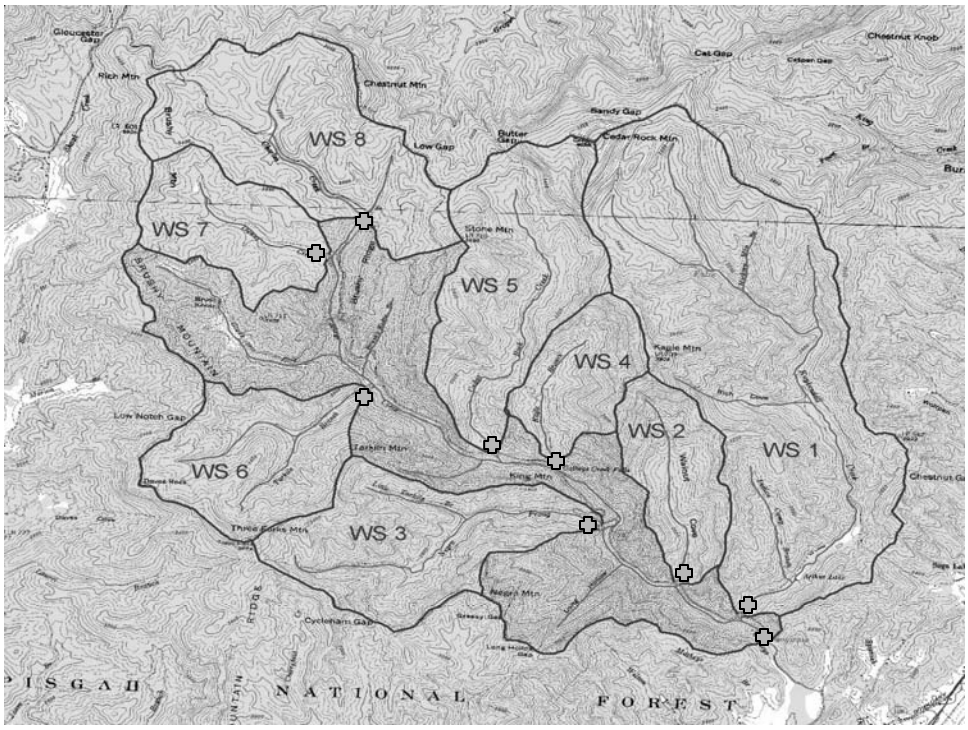

Figure 2—Sample sites (+), each in a distinct sub-watershed (WS), in the Cathey's Creek stream network: the lower drainage point on Cathey's Creek, Charles Creek (WS 8), Dunns Creek (WS 7), Tarkiln Branch (WS 6), Cedar Rock Creek (WS 5), Kagle Branch (WS 4), Negro Prong (WS 3), Walnut Cove (WS 2), and Kuykendall Creek (WS 1).

Six species of *Phytophthora—P. cinnamomi, P. citricola, P. citrophthora, P. gonapodyides, P. heveae,* and *P. pseudosyringae*—plus four other groups of isolates, which were morphologically and genetically distinct, were detected in the two stream networks. For each stream network, numbers of colonies and diversities of species varied among sample sites and between sample dates. Five species-groups were detected among 200 colonies recovered from the Davidson River network in September 2007, but only three of these were detected at the drainage point. In October 2007, nine species-groups were detected among 289 colonies recovered, and five species-groups were found at the drainage point. In the Cathey's Creek network, 155 and 219 colonies were recovered in June and October 2008, respectively, and seven species-groups were found at each sampling date. Three species-groups were detected at the drainage point in June, and five species-groups were found in October. The lower three tributaries in the Davidson River network had a higher mean density than that in the upper three tributaries (e.g., 41 vs. 10 colonies/900 ml, respectively). However, the upper three tributaries in the Cathey's Creek network had a higher mean density than that in the lower three tributaries (31 vs. 15 colonies/900 ml, respectively). With leaf baiting, three and two species-groups were detected at the drainage points on Davidson River and Cathey's Creek, respectively, during each sample period. All the species-groups found within a stream network were not detected at the drainage point. However, all of the species-groups present in the network that represented at least 9 percent of the total population were detected at this sample point. Based on this study, recovery of a species of *Phytophthora* at the drainage point is dependent upon the density of the population of that species in a forest stream network. Therefore, detection of a species present at a low population density may require more intensive sampling.

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