Sudden Oak Death: Monitoring Phytophthora ramorum in the North Central United States

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Introduction

In the mid-1990s, a new disease of tanoaks and coast live oaks appeared in northern California. Hundreds of trees appeared to be dying suddenly across the landscape, both in woodlands and in suburban properties around the San Francisco Bay area. In reality, infected large landscape trees did not die very suddenly; a few months to a few years could pass between infection and death.

However, once visible symptoms appeared, death occurred rather rapidly, hence the common name, Sudden Oak Death (SOD), which has been popularized in the press and most scientific literature. Interest in the disease increased progressively until it became obvious that SOD was becoming an epidemic of frightening proportions — thousands of trees were dying along the north and central California coasts.

In 2000, University of California scientists discovered that a Phytophthora spp. was consistently associated with the cankers that were characteristic of the syndrome in oaks. They soon realized that the same pathogen had previously been isolated and described from Viburnum in nurseries in the Netherlands, where it caused a branch-and-twig dieback. For this reason, the Dutch scientists had named the pathogen Phytophthora ramorum (ramorum literally means of the branches in Latin).

Over the next few years, it was recognized that the pathogen has a very large host list, which is not unusual for Phytophthora spp. The host list comprises a large number of woody shrubs that are characteristic of the understory in forests across the temperate zone, including plants such as viburnums, rhododendrons, honeysuckles, mountain laurel, and many others. On these plants, the pathogen only causes minor leaf spotting and twig dieback. The importance of these plants lies in the fact that they can act as conduits and reservoirs for the pathogen and that many of them are mainstays of the ornamental industry. (See: http://www.aphis.usda.gov/ppq/ispm/sod/usdasodlist.html.)

Movement of infected host plants through the nursery trade is therefore feared as a route through which the pathogen could potentially spread to areas where susceptible tree hosts are naturally distributed. Among the susceptible tree hosts are red oaks, including the northern red oak (Quercus rubra), a dominant species in the eastern mixed deciduous forests of North America. No natural infections of red oaks are known in the eastern United States. In Europe, however,
natural infection centers of *P. ramorum* were found in late 2003 in England and the Netherlands on two North American red oak species, northern red oak and southern red oak (*Q. falcata*), as well as on European beech (*Fagus sylvatica*) and horsechestnut (*Aesculus hippocastanum*).

This was a rather ominous development for Ohio, because the range of prevailing temperatures and relative humidity of the Midwestern region of the United States includes the range of climatic conditions found in England and the Netherlands. At least in principle, these findings demonstrated that there may be no climatic limitations to a potential spread of the pathogen to Ohio and the Midwest.

Fear that the pathogen could spread through the nursery trade was confirmed in dramatic fashion early in 2004. In March 2004, the pathogen was found in two large ornamental nurseries in southern California. At least one of the two nurseries regularly distributes nursery stock nationwide, including shipments to Ohio.

Indeed, on March 31, 2004, the state of Florida announced that *P. ramorum* had been found in a nursery that had imported stock from one of the infected nurseries in southern California. As of September 29, 2004, the total number of confirmed positive sites from trace-forward, national, and other survey finds was 160 in 21 states, including three residential finds — two in Georgia and one in South Carolina (these finds were connected with plants obtained by homeowners through the nursery/retail trade.) None of these finds were in Ohio.

The results of these surveys, conducted in commercial nurseries by the U.S. Department of Agriculture’s Animal and Plant Health Inspection Service (USDA-APHIS) in cooperation with state agencies, clearly underscore the high risk of movement of *P. ramorum* on infested plants around the country. A single escape from an infested nursery into surrounding woodlands or forests in areas potentially conducive to development of the disease, *e.g.*, on red oaks in Midwestern states, could be devastating.

Thus, large sections of U.S. forests are under potential threat of attack by *P. ramorum*. To date, however, except for an unconfirmed finding in New York state, Sudden Oak Death has been confirmed only in forests of California and Oregon.

In response to this threat, the U.S. Forest Service conducted a National Sudden Oak Death Survey in 2004. The purpose of the SOD survey was to examine forested areas around nurseries receiving potentially infected stock from California and Oregon, as well as other general forest sites, for the presence of *P. ramorum*. If the pathogen was found in these areas, eradication efforts would be initiated immediately in an attempt to destroy the pathogen before it becomes established.

### Methods

Our Ohio State University lab participated in the 2004 SOD survey, processing samples from sites in the North Central United States, including Ohio, Indiana, Illinois, Missouri, Iowa, and Wisconsin, based on U.S. Forest Service protocols (Figure 1). Two types of surveys were conducted — nursery perimeter surveys and general forest area surveys.

A nursery perimeter survey examined tree lines or woodlots located within a quarter mile of the nursery, while a general forest area survey examined a forested area that was more than a quarter of a mile from the nursery receiving stock. In addition, some forest area surveys were done with no relation to a nursery. Each...
site was surveyed with four 100-meter transects. Since the host list of *P. ramorum* is large, a subset of host species was chosen for this survey. Plant material with symptoms of leaf spots and/or twig dieback were sampled when present on *Acer* spp. (e.g., boxelder), *Aesculus* spp. (buckeyes), *Hamamelis* spp. (witch hazel), *Kalmia* spp. (mountain laurel), *Lonicera* spp. (honeysuckle), *Rhododendron* spp., *Vaccinium* spp. (blueberry), and *Viburnum* spp.

When present, phloem at the margin of oozing stem lesions was also collected from *Quercus* spp. (oaks). When symptoms were present, five leaves/shoots were collected from a tree/shrub and a maximum of 50 symptomatic leaves/shoots of a species per transect were collected. For each transect, the 10 most representative symptomatic leaves for each species were selected (Figure 2).

Bleeding cankers from oak trunks were also collected. A hand axe was used to chip away the outer bark to reveal the symptomatic inner bark beneath. A 5-x-5 cm section of the active canker margin was cut out and wrapped in plastic food wrap. All samples were then double-bagged and stored in an ice chest on sealed coolant and shipped to the lab for *P. ramorum* detection within 72 hours of collection.

Figure 1. Distribution of all sites directly surveyed by the Ohio State University Plant Pathology Lab during the summer of 2004. The geographic distribution of sites in Missouri and Wisconsin that were the origins of the samples the Ohio State lab processed was not available.

Figure 2. Immediately after collection, the samples were sorted, and the most representative leaves were selected for processing.
In the lab, samples (Figure 3) were processed for DNA extraction and PCR-based detection according to U.S. Forest Service protocols. One piece of necrotic tissue was cut from each suspect SOD-infected leaf using a cork borer. Ten pieces of tissue from a sample were combined for DNA extraction. Once DNA was extracted, PCR amplification of specific \textit{P. ramorum} DNA was attempted. Each PCR set contained positive controls of \textit{P. ramorum} DNA (to ensure that the PCR reaction was working) as well as negative water controls (to ensure that no \textit{P. ramorum} DNA was being amplified when not present).

**Results and Conclusions**

The 2004 SOD Survey in the North Central United States did not detect the presence of \textit{P. ramorum}. The following numbers of samples were processed — 89 from Ohio, 100 from Indiana, 75 from Iowa, 62 from Illinois, 75 from Missouri, and 219 from Wisconsin. Despite these reassuring results, further work is needed.

The 2004 survey inspected a subset of nurseries receiving potentially infected stock from California and Oregon, so there are more sites that need to be surveyed. Furthermore, in early December 2004, it was disclosed that the pathogen was detected in nursery samples in Connecticut.

The North Central United States apparently has the climatic conditions and host species for development of the disease on oaks, which means that the only missing element is the pathogen. If \textit{P. ramorum} escapes from infected nursery stock and becomes established in the landscape and forest, the effects will be devastating ecologically, aesthetically, and monetarily. Therefore, it is important to remain diligent in efforts to detect \textit{P. ramorum}. More nationwide surveys are already being planned for the summer of 2005.

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