What You Need to Know About *P. ramorum*

A plant physiologist and a plant pathologist at Purdue University explain how growers can minimize or stop the spread of sudden oak death (SOD) in their nursery stock.

In 1995, a quiet epidemic was killing the tanoaks *(Lithocarpus densiflorus)* in Northern California. Soon after, coast live oaks *(Quercus agrifolia)* and California black oaks (Q. *kelloggii)* were observed to be dying, as well. In the years that followed, scientists, foresters and others recognized that thousands of oaks were dead and dying, without knowing the culprit. In reality, most infected trees died slowly, with the disease requiring anywhere from a few months to even years between the time of infection and death. However, because death occurred shortly after the onset of visible symptoms, the common and dramatic name, sudden oak death (SOD), was established in the public, the press and the scientific literature.

It wasn't until 2000 that scientists at the University of California, Davis, identified a *Phytophthora* species that consistently was associated with the characteristic weeping cankers of SOD. The identification of the genus *Phytophthora* to a species level, however, is a painstakingly difficult task. Ultimately, the *Phytophthora* species they identified was a previously described species responsible for a twig blight on *Rhododendron* and *Viburnum* named *Phytophthora ramorum,* which first was identified in nurseries in the Netherlands.

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The naming of a pathogen allows for its identification and enables the establishment of quarantines. Within months of identifying P *ramorum* as the cause of SOD, Oregon had established a quarantine preventing the interstate trade of nursery products from California to Oregon. By 2001, the USDA had quarantined 12 California counties, restricting the movement of any nursery stock from those counties. Despite the quarantine, P *ramorum* was discovered soon after in Curry County, OR, and by 2004, it also was found in nurseries in western Washington and British Columbia, Canada.

Despite quarantines and the fact that many species of *Phytophthora* have broad host ranges, nurseries still were shipping plants with a limited understanding of this pathogen. This changed with the finding of P *ramorum* on *Camellia* in a premier California nursery in March 2004 and the realization that nurseries unknowingly had shipped infected plants across the US. By September 2004, the USDA finished tracing infected plants from nurseries and mail-order companies. After collecting trace-forwards, the USDA found 160 confirmed P *ramorum*-positive sites in 21 states, including three residential finds in the Southeast.

From the original six species (within three genera) susceptible to P *ramorum,* the host list now stands at more than 80 species (for updates, see: www.aphis.usda.gov/ppq/ispm/pramorum/). In addition to the previously named hosts, common nursery trade woody ornamentals that are associated with P *ramorum* include pieris, azalea, honeysuckle and lilac. On these plants, the pathogen causes only minor leaf spotting and twig dieback.

These symptoms, which are found in a number of species (not just oaks), resulted in an attempt by the nursery industry to rename the disease to the more correct ramorum canker and blight. The name change did not catch on, which may not be a bad thing. Although correct, this less dramatic name certainly will not get a homeowner's attention in a way "sudden oak death" can.

The SOD outbreak in Oregon was linked to the movement of infected host plants through the nursery trade. Prior to, and concurrent with, the SOD outbreak in the US, P *ramorum* was found in England and the Netherlands, infecting both the Southern red oak (Q. *falcata)* and Northern red oak (Q. *rubra),* in addition to horse-chestnut *(Aesculus hippocastanum)* and European beech *(Fagus sylvatica).* Concern about the potential spread of this pathogen east of the Mississippi River is well-founded, although no natural infections of red oaks or other species are known in the Eastern US, based on two years of the National Sudden Oak Death Survey performed by the USDA Forest Service in forests adjacent to nurseries throughout the Midwest. Despite the lack of infections, the Midwest still is at risk for P *ramorum* establishment.

In addition to forest surveys, nursery surveys are performed by state departments of agriculture. If the pathogen is found, eradication efforts will be initiated immediately in an attempt to destroy the pathogen before it becomes established.

Despite the surveys and strict monitoring at both the shipping and receiving ends, accidental P *ramorum* introductions continue throughout the US. This past summer, Indiana became the first state in the Midwest to have a positive trace-forward. Around the same time, a positive trace-forward was identified in Maine. These incidents have caused growers in the Midwest and Eastern states to ponder the questions "How are plants tested and confirmed?" and "What happens if a plant tests positive in my nursery?"

Nursery plants that have been infected by *Phytophthora ramorum* are destroyed by incineration (burning to ash). *P. ramorum* has been identified as the cause of sudden oak death. In September 2004, the USDA found 160 confirmed *P. ramorum*-positive sites in 21 states, including three residential finds in the Southeast.

Looking for *P. ramorum.* Screening for P *ramorum* occurs on three levels. There is a national survey, in which a number of nurseries in each state that have a large volume of nursery stock from the West Coast are monitored routinely for the disease. These nurseries are considered "high risk" for introduction of the disease. There also are routine nursery inspections. These inspections are part of the certification process in most states. The inspections are conducted to identify any pests and diseases that may be present. In the case of P *ramorum*, inspectors specifically will check material that has come from the West Coast. Finally, there is trace-forward screening. This screening is done in conjunction with West Coast nurseries and occurs when material has been shipped from a nursery that has been declared positive for P *ramorum*.

In addition to these screens performed by nursery regulatory bodies at the state and national levels, the forest service also conducts screens around the perimeter of selected receiving nurseries. Sites that have received material from infected nurseries are monitored carefully for P *ramorum*.

Testing plant samples. In the beginning of this article, we discussed the several-year lag between the isolation, determination of pathogenicity and, ultimately, the identification of P *ramorum*. The identification to the genus *Phytophthora* is fairly simple, but the reliable identification to species is much more difficult. To identify *Phytophthora* quickly and reliably, an antibody-based test called ELISA (enzyme-linked immunosorbent assay) is used. ELISA detects the presence of *Phytophthora* through the use of antibodies and/or antigens in a sample. The use of ELISA is a quick screen for *Phytophthora* determination, and it is by no means conclusive.

ELISA-positive reactions then are subjected to PCR (polymerase chain reaction). PCR is a molecular technique that allows a small amount of DNA to be copied exponentially, but in a very specific way. This specificity is determined by the types of primers used to perform PCR. DNA is extracted from the sample suspected of P *ramorum* infection (in a sample that already has been confirmed for the presence of a species of *Phytophthora* by ELISA). The Animal and Plant Health Inspection Service (APHIS) branch of the USDA uses nested PCR, which amplifies a large piece of DNA in a first reaction, then amplifies a smaller, nested fragment that is specific only to *P ramorum* in a second reaction.

Phytophthora species other than P *ramorum* are common problems in the nursery, so it isn't surprising that any nursery tested will have ELISA-positive tests for *Phytophthora.* These samples then are further tested to determine conclusively if *P. ramorum* is the culprit. Most samples are negative, but what happens if a sample in your nursery tests positive for P *ramorum?*

After identifying *ramorum.* The protocols for dealing with a preliminary positive identification of *P. ramorum* on a nursery site are similar to those for any identified pest or disease. The objective of the response is to keep infected plant material from being transported to other locations and to destroy those plants that are carrying the disease to stop any spread to other plants. There are several factors that will dictate how extensive the response must be. Understanding these factors can help growers minimize losses.

The regulatory response is set in action when *P. ramorum* has been positively identified during one of the surveys described previously or by other means. The protocol for dealing with a positive identification is regulated by the state and/or federal government. Positive identification is reported to the state plant health

Besides incineration, plants infected by *Phytophthora ramorum* can be destroyed by deep burial (placed in plastic bags and buried at least 2 meters below ground) or steam sterilization (heated to 100°C for 30 minutes).

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director or the state regulatory official. These officials are responsible for securing the nursery site, destroying infected or potentially infected material, making any public announcements and notifying facilities that may be impacted by trace-forwards or trace-backs. The trace-forward involves locating plants that have been shipped from the quarantined nursery. This may include retail garden centers or plants installed in a landscape. In a trace-back, a nursery or nurseries from which plants infected with P *ramorum* have originated will be notified and inspected for further spread of the disease.

All plants that are infected or potentially infected must be destroyed. After carefully surveying the nursery, the inspector will designate a destruction block. Within the USDA's Official Regulatory Protocol for Wholesale and Production Nurseries Containing Plants Infected with *Phytophthora ramorum,* a destruction block is defined as a "contiguous block of [host plants] containing one or more plants known to be infected with P *ramorum."* The block is considered contiguous until there is a 2-meter break with either no plants or no host plants.

After P *ramorum* has been positively identified, the inspector can order a hold on any material that is deemed to be a risk for spread of P *ramorum.* The size and number of destruction blocks depends on several factors. If adjacent plants are either the same species or another host plant for P *ramorum,* they need to be destroyed. Also, because P *ramorum* is spread by water, plants that are in the pathway of runoff water from infected plants also are subject to destruction. All equipment within the nursery must remain in place until proper disinfestation occurs, and the movement of soil that potentially is infected also is restricted.

Following the initial quarantine, the inspector will survey the nursery and perimeter. All host plants for P *ramorum* will be inspected, and those showing symptoms will be tested for the disease. All plants within 10 meters of destruction blocks will be held for the quarantine period, and unhealthy tissue will be sampled. The perimeter of the nursery will be surveyed to determine if the disease has spread to the environment immediately outside the nursery or if the original source of the infestation was the surrounding environment. Host plants within 100 meters will be surveyed.

After the surveys have been completed and the destruction block(s) established, the infected plants(s) will be destroyed by incineration (burning to ash), deep burial (placed in plastic bags and buried at least 2 meters below ground) or steam sterilization (heated to 100° C for 30 minutes).

After the surveys have been completed, a 90-day quarantine period begins. Any nonhost plants that were within the destruction block and any other host plants in the nursery are restricted during this period. There is a catch with the 90-day period, however: This minimum quarantine length only applies if the 90 days following the survey are during a period when climatic conditions are conducive to the spread and infection of the disease. If the climatic conditions are not conducive, it is impossible for inspectors to determine if the nursery is disease-free. Therefore, the inspector may extend the quarantine period if necessary. Inspectors will evaluate plants at least twice: approximately halfway through the quarantine period and near the end of the quarantine period. A final quarantine-release survey will be conducted before plants are removed from quarantine.

The inspector also will initiate trace-back and trace-forward investigations. It is important to note that the quarantined nursery is responsible for maintaining sufficient information to carry out the trace-forward. This may be straightforward if the nursery is a production nursery selling to relatively few clients. However, in the case of a retail nursery, keeping track of every customer is not as easy. It is beyond the scope of this article to discuss the various ways that businesses can track their customers, but it is something that every nursery is responsible for. Failure to identify customers who have purchased plant material from your nursery can result in regulatory action against your business.

According to APHIS, the nursery will be released from regulatory control if the following three conditions are met:

- There are no additional detections of P *ramorum* in nursery stock based on USDA APHIS-approved plant inspection, sampling and testing protocols for the preceding quarantine period;
- Water, soil and growing media also have tested negative for P *ramorum* based on USDA APHIS-approved sampling and testing protocols for the preceding quarantine period; and
- The quarantine-release survey is negative for P *ramorum.*

Precautions you can take. There is no doubt that the identification of P *ramorum* in your nursery can have a devastating effect on your business. A nursery using recirculating water that tests positive for P *ramorum* could conceivably lose its entire inventory, and some large nurseries on the West Coast have lost millions of dollars due to infected plants. However, with a good understanding of the biology

of the pathogen and a familiarity with the regulatory response, business owners and managers can do several things to minimize losses should this pathogen be identified. Protecting against P *ramorum* infestation includes exclusion of the pathogen, implementing best-management practices that limit the spread of accidental introductions and constant scouting for the disease.

Exclusion —When purchasing plants that are hosts for *P ramorum*, only purchase from certified nurseries. California, Oregon and Washington all have certification programs in place. Stock routinely is checked for the presence of P *ramorum*, and only clean material is certified. Check with your state department of agriculture or other regulatory body for acceptable certification programs. In most states, it is illegal to purchase stock that has not been certified by a duly authorized state or federal official. However, if your state allows purchasing from noncertified nurseries, it is extremely important to have the material tested and to keep it segregated from other nursery stock. All incoming stock should be visually inspected for symptoms of P *ramorum*. If symptoms are present, the plants should be tested as soon as possible for the presence of P *ramorum*.

Best-management practices — As discussed earlier, the goal of the regulatory agencies is to isolate infected or potentially infected plants and destroy them. Your ability to manage your stock can have a great effect on the extent of the regulatory response. An updated set of best-management practices can be found at the California Oak Mortality Task Force Web site: http://nature.berkeley.edu/comtf/html/nursery_best_mgmt_practices.html.

One best-management practice that will minimize losses is to avoid large blocks of host plant species. In the case of an infestation, separate host species with blocks of nonhost species or open spaces to minimize the number of plants that will need to be destroyed. Of course, it is not possible to keep each species of plant separated from all others; however, there are some things you can do. For example, all material imported from the West Coast can be maintained in a restricted area. Potential host plants also can be isolated from each other (keep in mind the 2-meter zones around destruction blocks). For example, if groups of *Viburnum* that are positively identified are located next to a block of azaleas, it is likely that all those plants would have to be destroyed. If, however, that same block of *Viburnum* was next to a block of *Buxus*, the damage would be limited.

Isolation is the best solution

Imagine two scenarios. In the first scenario, *Phytophthora ramorum* is positively identified in a single plant of 100. The plants arrived at the nursery within the previous week and have been kept separate from other host plants. In this case, these 100 plants would be destroyed, and if upon further inspection the rest of the nursery was found to be clean, the nursery would continue business as usual.

In the second scenario, P *ramorum* is identified in a plant that was grown from a liner received three years prior to identification. In the meantime, the liners were distributed throughout the nursery and intermingled with other potential host species. In this case, the entire stock of this nursery may have to be destroyed. Thus, if host plants can be isolated, nurseries can avoid potentially huge losses.

Understanding that *P. ramorum* is spread by water will guide management decisions. Knowing the host plants that can be infected by *P. ramorum* will aid in stock management and avoid unnecessary worry.

Ornamental plants on the property, such as landscape plants around buildings, that are hosts may be infected and, in turn, infect a large number of plants moving through the nursery. Alternatively, the plants could be considered sentinels, allowing you to determine if the problem exists. *Viburnum*, for example, will succumb in a matter of weeks following infection by *P ramorum*. Select permanent landscape plants carefully. They play an important role in your business, but they also may lead to spread of the disease.

Because P *ramorum* (like other *Phytophthora* species) is spread by water, careful water management is essential to limiting the effect of outbreaks. Drainage is critical: Be sure that drainage of plant water runoff is directed away from other plants and that pooling does not occur. Many nurseries use recirculating water systems in which water applied to plants is recycled through a common retention pond. These systems are excellent in terms of water efficiency. However, they also can contribute to the spread of diseases, such as *P ramorum*, if the water is not properly treated to eliminate disease. Recirculating water systems should be maintained to ensure they are working properly. Filters should be changed regularly, and components, such as ultraviolet water purifiers, should be checked and maintained on a regular basis. Failure to do so could result in the entire nursery being quarantined and all stock being destroyed.

Be sure that your propagation and potting practices minimize the risk of introduction or spread of the disease. Use cuttings from plants that have not shown any symptoms for the past several years. Properly disinfect all pots and tools, and never reuse soil if possible. Properly dispose of all waste plant material and soil.

Scouting — As with all aspects of nursery management, knowledge is key. The more you understand about pests and diseases, the more able you are to avoid problems or deal with them appropriately when they arise. Be sure you can identify P *ramorum* symptoms, and constantly be on the lookout. A great pictorial guide can be found at: www.ppdl.purdue.edu/PPDL/pubs/SOD_pictorial_guide.pdf.

The symptoms of P *ramorum* are similar to other diseases, so you should know as much as you can about the probability of a symptom being related to P *ramorum*. Understanding that P *ramorum* is spread by water will guide management decisions. Knowing the host plants that can be infected by P *ramorum* will aid in stock management and avoid unnecessary worry.

It may not be possible to stop the spread of P *ramorum* or other infectious diseases forever; however, using effective exclusion techniques, best-management practices and constantly scouting to destroy accidental introductions as soon as possible will slow the spread. Minimizing the risk of introduction and managing stock in such a way to prevent spread pays for itself when you consider the potential losses in the hundreds of thousands to millions of dollars that can occur should the pathogen be found in your nursery.

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