GROWING KNOWLEDGE

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Surface water sources are often used for irrigating plants. PHOTO COURTESY OF OREGON STATE UNIVERSITY

Testing the waters

How to test your irrigation system for the presence of *Phythophthora*

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PHYTOPHTHORA is a fungus-like organism — called a water mold — that can infect a wide variety of nursery plant species. One of the ways it can spread is through irrigation water.

Depending on size, a nursery can demand between several hundred to several hundred thousand gallons of irrigation water per day. Several sources of water may be used to meet these irrigation demands, including surface water (rivers, streams, canals, lakes, ponds, reservoirs), well water, rainwater, municipal drinking water, and recycled runoff water.

Of these, only rainwater, well water, and municipal water are free of pathogens. Growers should assume that all other sources are contaminated and disinfest them before use.

Alternatively, people can test their water sources at frequent intervals and treat only if necessary.

Once pathogens gain entry into the nursery, they can spread through the irrigation system, infect plants, accumulate in the runoff water, and establish in the water storage reservoirs. Disinfesting contaminated water before it is used for irrigation is essential for breaking this cycle.

Phytophthora

There are more than 100 *Phytophthora* species; some are host-specific, while others can cause disease on hundreds of plant species. All require water to complete their life cycle.

Phytophthora species cause foliar blight, stem canker, shoot dieback and root rot on ornamentals, native plants, forest trees and agricultural crops worldwide. Many nursery plant species are highly susceptible, such as azalea, rhododendron, boxwood, and many conifer species. A host list is available on **tinyurl.com/y6k25dah.**

The plant nursery environment is optimal for growth and proliferation of *Phytophthora*. Oospores and chlamydospores are capable of long-term survival in soil or infected plant debris.

Under wet conditions, *Phytophthora* sporangia release numerous small, swimming spores called zoospores, which can infect plants and cause disease (Fig. 1). The zoospores not only survive and propagate in surface water but also can be delivered throughout the nursery in irrigation water.

Testing with bait

Baiting is a relatively simple technique that uses susceptible plant parts as "baits" to attract *Phytophthora* spp. This method selects for live, active zoospores, which can swim toward and infect the bait.

Growers can use rhododendron leaves or hard, unripe pears (such as Bartlett or d' Anjou) as bait to detect *Phytophthora*, as they are susceptible to many species of *Phytophthora*. These baits can also capture several *Pythium* species, which are closely related to *Phytophthora* and cause damping-off of seedlings and stem cutting rots. There are two basic ways to bait: in a resealable bag (indoors), or in the water body itself (outdoors).

Baiting indoors

Collect a quart of water in a clean, soap-free container from near the surface of the water that will be tested. Pour half the water into each of two one-gallon resealable bags labeled with the date and sample name. Place a rhododendron leaf or a unripe pear in each bag. Be sure to choose leaves or fruits without spots, blemishes or bruises; they should be free from pesticides that could interfere with the test.

It is always a good idea to place a few leaves in bags with tap water or distilled water as a negative control.

Allow the bags to sit for seven days at 65–75 F, out of direct sunlight.

Then, remove the leaves or the pears and examine them for chocolate-brown spots, known as lesions, on rhododendron leaves or brownish red circular lesions on pears. If the pear has been sitting upright, you can sometimes see a "bathtub ring" of lesions around the pear made by zoospores (Fig. 2).

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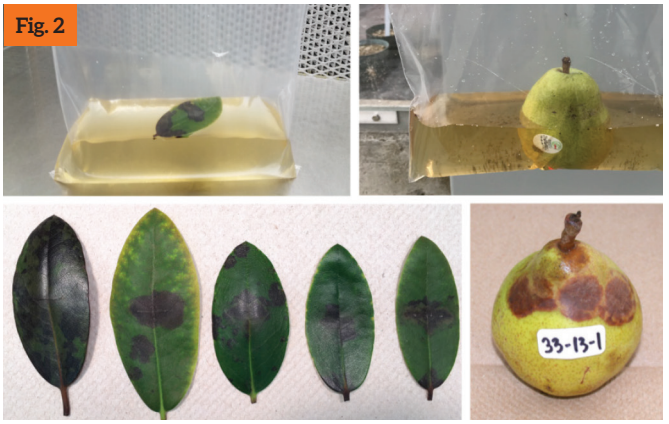
Fig. 1: Top left: *Phytophthora sporangium* releasing zoospores. PHOTO COURTESY OF FRED SCHWENK **Top right:** Foliar blight symptom on rhododendron plants. Bottom: Rhododendron root rot caused by *Phytophthora*. PHOTO COURTESY OF OREGON STATE UNIVERSITY

Fig. 2: Top left: Baiting of water with rhododendron leaves. **Top right:** Pear baits. **Bottom:** Brown lesions caused by *Phytophthora* species. PHOTO COURTESY OF OREGON STATE UNIVERSITY



Spots made by *Phytophthora* are firm to the touch; if they are soft and mushy, they are likely caused by a soft rot organism; not *Phytophthora*. For more information, watch our tutorial on baiting for *Phytophthora* at **tinyurl.com/yx8t8y5m.**

Baiting outdoors

To bait outdoors, make a mesh bag to hold the leaf baits. Plastic window screen material works well for this purpose. Some mesh bags are made with sleeves for individual leaves, as shown (Fig. 3). Attach the mesh bag to an air-filled, plastic milk jug to act as a float, as the greatest number of zoospores will be near the water surface. Secure the mesh bag with a nylon rope, so you can cast the bag out into the water and then retrieve it seven days later.

Fig. 3: Rhododendron leaves in a mesh bag showing lesions after baiting outdoors. PHOTO COURTESY OF MARIANNE ELLIOTT

Remove the leaves from the bag and examine them for dark, chocolate-brown lesions. The outdoor baiting works well when the water temperature is between 48 F to 71 F.

If *Phytophthora* is present in water, zoospores will swim toward the surface of water surrounding the bait, colonize it and develop dark brown lesions. If the tested water is contaminated with a high amount of the pathogen, then brown lesions may appear within two to three days after baits are suspended in water.

The appearance of lesions on the bait is a good indication that *Phytophthora* species are present, but this should be confirmed.

Detecting *Phytophthora* after baiting

To confirm the presence of *Phytophthora*, remove a small piece of the bait lesion and test it with a commercially available diagnostic kit for *Phytophthora* species (Fig. 4).

Phytophthora diagnostic kits are based on polyclonal antibodies for detection of multiple *Phytophthora* species. The kits are rapid, easy to use and relatively inexpensive. For example, the Pocket Diagnostics® *Phytophthora* kit costs about $8 each when purchased in boxes of 50 kits. It takes less than 10 minutes to conduct the test. For details visit **www.pocketdiagnostic.com/online-shop** or **www.potadaho.com/test-kits.**

These kits are designed for genus-level detection of *Phytophthora* species, but will not identify the particular species. To watch our online tutorial on how to use rapid test kits for *Phytophthora*, visit **tinyurl.com/yy8s5vfh.**

If the *Phytophthora* test is positive and you need to know what species is present, send the bait for additional testing by a university plant diagnostic lab. (Please note that it is much easier to send leaf baits rather than pears through the mail.) Be sure to contact the lab in advance to make sure they can handle the sample.

Plant diagnostic labs can attempt to identify the species by growing it in a »

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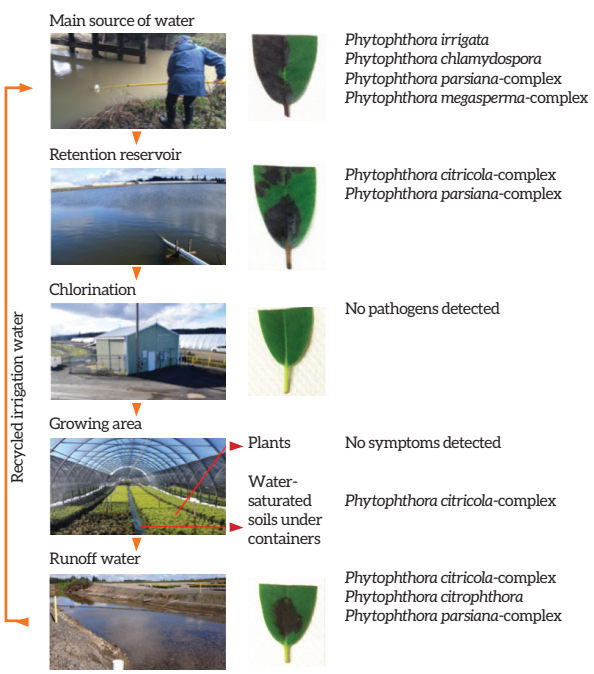
Fig 4: Left: Lateral flow device showing positive results for *Phytophthora* detection using the Pocket Diagnostics *Phytophthora* rapid test kit. **Right:** Negative results of *Phytophthora* test. PHOTO COURTESY OF OREGON STATE UNIVERSITY
Fig. 5: Chlorination of irrigation water reduces *Phytophthora* contamination. PHOTO COURTESY OF OREGON STATE UNIVERSITY

Fig. 4

Fig. 5



petri dish containing an agar nutrient medium selective for *Phytophthora*. Identification can be based on microscopic features or DNA-based techniques. For most growers, genus-level identification is enough to demonstrate that their water is contaminated and requires treatment.

Taking steps to disinfest water

Irrigation water treatment can minimize the spread of *Phytophthora* and help reduce losses. There are several methods for water disinfestation with chemical, physical and biological modes of action. Choose which method to use based on the mode of action, the volume of water to be treated, installation and operational costs involved, space requirements, and safety and environmental concerns.

Refer to the "Waterborne solutions tool" that summarizes water treatment technologies used to control plant pathogens at the CleanWater3 website: **http://cleanwater3.org/growertools.asp.**

Successful reduction of *Phytophthora* in irrigation water

A large container nursery in Oregon recycles 90 percent of its irrigation water; it succeeds in preventing *Phytophthora* infestation of its irrigation water by treating it first with sodium hypochlorite or calcium hypochlorite.

We collected water samples from several different steps along the irrigation water pathway, and baited the water samples with rhododendron leaves to see if *Phytophthora* was present. We then used a DNA sequencing technique to identify the *Phytophthora* species that were on the bait leaves. The diagram (Fig. 5) illustrates results of baiting irrigation water in the month of September 2015.

The main source of water for the nursery was a creek. The creek was infested with four *Phytophthora* spp., including two pathogenic species complexes, *P. parsiana* and *P. megasperma*. (A complex is a group of closely related species that cannot be distinguished with our method of sequencing.)

The creek water was pumped to a retention reservoir where we detected two species complexes: the *P. parsiana* complex from the creek, and the *P. citricola* complex from another source, likely the runoff water.

Plant disease diagnostic labs

Oregon State University Plant Clinic
http://plant-clinic.bpp.oregonstate.edu
- Baiting water for *Phytophthora* $75
- ELISA assay for *Phytophthora* $45
- Testing water via filtration for *Phytophthora* or *Pythium* $110

The Pullman Plant Pest Diagnostic Clinic at Washington State University
www.plantpath.wsu.edu/diagnostics
- Baiting water for *Phytophthora* $40

Plant Diagnostics Laboratory at University of Massachusetts
www.ag.umass.edu/services/plant-diagnostics-laboratory
- Testing irrigation water for *Pythium*, *Phytophthora*, and *Rhizoctonia* $50

Florida Extension Plant Diagnostic Clinics at University of Florida
https://plantpath.ifas.ufl.edu/extension/plant-diagnostic-center
- Baiting for *Pythium* and *Phytophthora* $65
- Immunostrip test for *Phytophthora $50*

Plant Disease Diagnostic Clinic at Cornell University
www.plantclinic.cornell.edu
- ELISA assay for *Phytophthora* $70
- Immunostrip test for *Phytophthora* $60
- PCR for *Phytophthora ramorum* $50

Plant Diagnostic Lab at North Dakota State University
www.ag.ndsu.edu/pdl/services-and-fees
- Immunostrip test for *Phytophthora* $45

Plant Disease Diagnostic Clinic at University of Wisconsin-Madison
www.pddc.wisc.edu/services-fees
- ELISA assay for *Phytophthora* $35
- Dip stick serological test for *Phytophthora* $8

Plant Disease Diagnostic Lab at Texas A & M University
https://plantclinic.tamu.edu/fees
- ELISA assay for *Phytophthora* $20

From there, the water was filtered and chlorinated, with a target concentration of 2 ppm and a 10-minute contact time. No *Phytophthora* species were detected in the chlorinated water, which was then used for overhead irrigation in the growing areas of the nursery. Although the irrigation water was clean, and the plants did not show any symptoms of *Phytophthora*, we did detect *P. citricola* complex in the soil/crushed rock material underneath the containers.

Soil in nurseries is commonly infested by *Phytophthora* species, which can survive for years in bits of plant debris that infiltrate the soil and crushed rock. It is very difficult to disinfest contaminated soil.

Runoff water from these growing areas, after contacting the contaminated soil, was found to harbor *P. parsiana* complex, *P. citrophthora*, and the *P. citricola* complex. Pumping the runoff water back into the retention reservoir carried these *Phytophthora* species with it. Fortunately, the subsequent chlorination treatment prevented *Phytophthora* from entering the irrigation water, so the contamination cycle was broken. Following this example, test irrigation water along its flow path at regular (monthly) time intervals.

By routinely testing the water with baiting, a grower can assess the risk for waterborne *Phytophthora*, and can implement preventive measures, if necessary.

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