Diagnostic Tip of the Month

Baiting Soil Using Leaf Pieces to Detect *Phytophthora* Species

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Plant disease clinics frequently have clients that suspect their soil contains root rot pathogens and would like to have it tested. Since Phytophthora species are common, virulent pathogens, effective soil testing techniques are important diagnostic tools. Soil baiting and direct soil plating are two techniques that can be used to detect multiple species of Phytophthora. The principle of soil baiting is that by flooding soil with water, zoospores will be released from propagules in the soil, swim upward because they are negatively geotropic, and infect the leaf pieces used as baits. Infected baits then can be plated on a medium selective for Phytophthora species.

Figure 1

Baiting has several advantages over direct plating. First, a larger quantity of soil can be tested, which increases the likelihood of detecting a pathogen present at a low population density. Secondly, homothallic species that survive as dormant oospores are more likely to be detected by baiting than by direct plating (1).

Various plant parts have been used in soil baiting techniques. In our clinic, we've used leaf disks from silver dollar tree (*Eucalyptus cinerea*) for many years, but leaves from other plant species also work as well or better. Ferguson and Jeffers (1) found leaf disks from *Camellia japonica* to be the most effective baiting tissue. Other leaf baits that performed reasonably well in their experiments were intact shore juniper needles, eucalyptus leaf disks, and intact Japanese holly leaves. One should choose a bait that is readily available, convenient to use, and susceptible to the target pathogen. Currently, Dr. Jeffers' lab routinely uses leaf pieces from both *C. japonica* and *Rhododendron catawbiense* (personal communication) when looking for *Phytophthora* spp. in soil samples.

The technique is as follows. Fill a small beaker with soil from the sample and place it in an open container. The soil should be about one centimeter deep. Add distilled or de-ionized water in a ratio of 1 part soil to 2 parts water. There should be at least one centimeter of water above the soil, but not more than two. In the 10.5 x 10.5 cm container pictured in Figure 1, 50 cc of soil flooded with 100 ml of water will provide these conditions. Obtain enough leaf tissue to float a number of cut or intact pieces on the water surface, as in Figure 1. The number of baits to use will depend upon how long you want to keep leaf baits available for infection and how many plates you want to examine. Twelve baits will provide enough tissue to prepare one plate after 24 hours and another after 48 hours. We use five baits per plate but float six in case one sinks. A 72-hour period also can be used, but the amount of contaminating fungi, such as *Pythium* spp., can be a problem—occasionally overwhelming the developing

Phytophthora species. Jeffers and Ferguson (1) found that detection was better when leaves were left in the bait

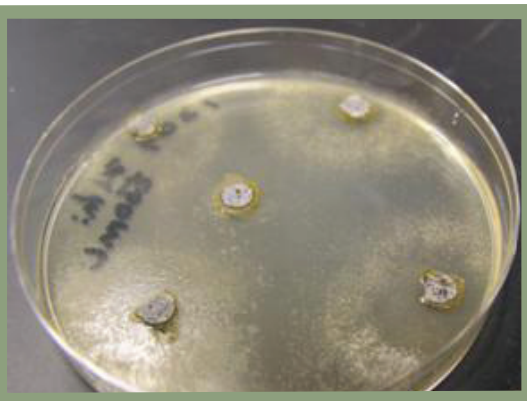

Figure 2

box longer (up to 72 hours)—particularly with soils that have a low population density, but the amount of contamination also was greater. Therefore, use enough baits to plate five at 24 hours and five more at 48 or 72 hours. The leaf pieces may be surface-sterilized prior to placing in the container, but this isn't absolutely necessary. If the plants used to supply baits are growing where propagules of *Phytophthora* spp. might splash onto the leaves from the soil, then surface sterilization is advisable.

Hold baited soil samples for 24 to 72 hours at room temperature. Then, at 24 hour intervals remove baits from the container and blot dry on paper towels. Place five baits onto PARPH medium (2) and incubate in the dark at 20-25°C. The baits should be gently slid into the medium, not just placed on the surface. After 2 or 3 days, examine the plates for growth of Phytophthora species, as shown in Figure 2; if obvious growth has not occurred, return plates to the incubator for up to 7 days. If a colony has a macroscopic resemblance to *Phytophthora* spp., the entire plate can be placed on the stage of a compound microscope to check for mycelium characteristic of Phytophthora species (Figure 3) and reproductive structures such as sporangia (Figure 4), chlamydospores, and oospores.

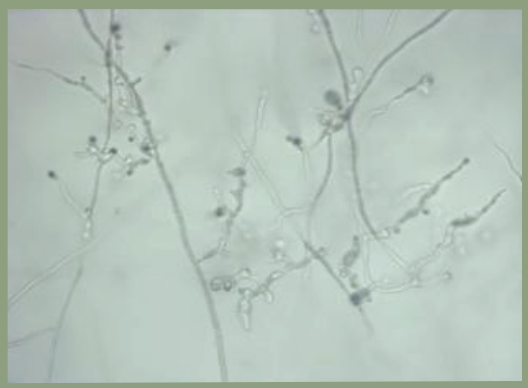

Figure 3

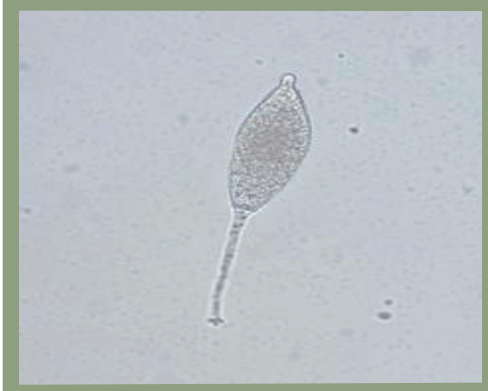

Figure 4

References
1. Ferguson, A.J. and Jeffers, S.N. 1999. Detecting Multiple Species of Phytophthora in Container Mixes from Ornamental Crop Nurseries. Plant Disease 83: 1129-1136.

2. Jeffers, S.N., and Martin, S.B., 1986. Comparison of two media selective for Phytophthora and Pythium species. Plant Disease 70:1038-1043.