

# Diagnostic Tip of the Month

## Diagnostic Tip of the Month: Oak Wilt Testing

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Figure 1 (top). Branch showing vascular staining. Figure 2 (bottom). Vascular staining may be deep in the wood. (Photo Nancy Pataky, University of Illinois)

The causal fungus of oak wilt is *Ceratocystis fagacearum*; but it is the anamorph, *Chalara*, that we are able to isolate in the lab.

This fungus is not always uniformly distributed in the tree, so it is essential to obtain branches that show vascular staining. Often this appears as brown streaks in the outer sapwood as shown in Figure 1. In some cases the staining may be deeper in the wood, as shown in

Figure 2. The pith discoloration in this image is not related to oak wilt.

*Chalara* can be isolated on a conventional mix of potato dextrose agar. Acidifying the agar will help reduce bacterial contaminations. This is helpful because plates need to incubate at room temperature for 7-10 days before a positive can be confirmed.

Branches to be tested must show vascular streaking and must be alive. Ask for branch sections that are 8-10 inches long. You must be able to hold firmly one end of the branch as you flame sterilize the

other end. Do this by dipping the branch section in a tall, narrow container of alcohol and then touching it to a flame. Sometimes the bark does not want to quit burning, so you may need to put out the flame by sharply waving it in the air under the transfer hood.

Branch sections that are at least as thick as but no larger in girth than your thumb, work best. Larger limbs have thicker bark, making it more difficult to remove the bark without contaminating the sapwood.

Next, flame sterilize a budding knife or other sturdy, sharp knife. Use the knife to peel off the bark, revealing the stained sapwood. If staining is not visible, remove sapwood until you can see the staining. Re-flame the knife. Next, take small chips of wood, no more than 1/8 to 1/4 inch thick (Figure 3) from this stained area and place chips into the agar so that half of each chip is in the agar and the other half exposed to the air in the plate.

Find a technique that allows you to hold the sample and open the plate only enough to insert the wood chip as seen in Figure 4.

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Figure 3. Taking chips from discolored wood and placing it in agar. (Photo Nancy Pataky, University of Illinois)

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Avoid placing the sample down, and avoid leaving the lid on the table surface. Prepare at least four plates so that you can keep checking for sporulation without contaminating all of your cultures.



Figure 4. Open the plate only enough to insert the wood chip. (Photo Nancy Pataky, University of Illinois)

Observe the culture plates regularly for the development of a dark green to brown colony as in Figure 5. Confirmation of a positive isolate can be made when the spores of *Chalara* can be found. These spores are rectangular in shape and occur in chains that emerge from the end of the conidiophores.

Since the spores disperse easily in water, use a tape mount technique to see the spores in intact chains. Touch the tape to the older part of a colony, and place on a drop of water on a microscope slide to be viewed on a compound microscope.

There is one bit of advice that we learned the hard way. The high temperatures of mail trucks will work to kill or slow the



Figure 5. Dark brown to green colonies of *Chalara* on agar. (Photo Nancy Pataky, University of Illinois)

oak wilt fungus. If you are seeing staining in your samples but are not isolating the oak wilt fungus, suggest that the client submit wood samples packaged between frozen disposable ice packs.

## Diagnostics Subcommittee Update

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The Diagnostics subcommittee held a conference call on June 14, 2007. During this meeting a number of issues were addressed. Please refer to the diagnostics subcommittee web page of the [NPDN web site](#) for complete minutes of this meeting (login and password required).

Topics of discussion included:

- Updated Soybean Rust SOP version 2.0 now available.
- Laboratory Accreditation Update.
- Creation of Leadership Subcommittee.
- Diagnostician Website Access to Committee Pages.
- Legume Virus PIPE SOP now available and distributed.
- IT-Diagnosticians Meeting Plans- November 28-29, 2007.

The next meeting will be held on **July 12, 2007**.

## Diagnostic Update