

# Diagnostic Updates

## Diagnostics Subcommittee Update

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The NPDN diagnostics subcommittee did not hold the scheduled conference call on December 13, 2007 due to severe weather across much of the country. However, the subcommittee members have been working via email on the planning of upcoming Beltsville training.

Information about the training and those participating will be posted on the subcommittee web page. Please refer to the [NPDN web site](#) for past meeting minutes and information about the training (login and password required).

The Beltsville-NPDN Diagnostician Training will be held during the weeks of February 4, 2008 through April 7, 2008.

Members of this subcommittee polled their regions looking for interest in a number of training sessions. When the response came in, members of the NPDN were interested in 56 training slots!

The current plan is for Laurene Levy and her staff at the USDA, APHIS, PPQ, CPHST, National Plant Germplasm and Biotechnology Laboratory in Beltsville, Maryland to conduct training sessions that cover a number of identification

technologies used to identify significant pathogens.

Currently planned are one session that will cover the pathogens that cause Citrus Greening, two sessions that will cover the Potato Cyst Nematode, two sessions on Plum Pox Virus, and three sessions reviewing and presenting technologies for the identification of *Ralstonia solanacearum* Race 3 Biovar 2. As the plans are finalized, the schedule will be posted on the web page.

The next conference call will be held on Thursday, January 10, 2008.

## Diagnostic Tip of the Month: Carrot Bait for *Thielaviopsis basicola*

Meg Williamson  
Diagnostician  
Clemson University Plant Problem Clinic

Black root rot, caused by *Thielaviopsis basicola*, can be difficult to diagnose by microscopic examination of roots,

especially those from woody plants. A carrot bait can be used to accomplish this.

We use a bait technique in which a segment of carrot is cut longitudinally and the roots are placed between the two pieces. As opposed

to the method of distributing roots over carrot discs, this provides an incubation environment with more moisture.



Figure 1. Cut carrot with well in one half.  
Photo by Meg Williamson.

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Step by step instructions follow.

1. Wash roots well and surface sterilize for one minute in 10% bleach. Pour off bleach solution, replace with sterile water and allow the roots to soak for two or three minutes. Pour off water and dry roots on paper towels, blotting well. Using sterile scissors, cut roots into small pieces about 5 - 10 mm long.

2. Cut a carrot segment and surface sterilize in 10% bleach for at least one minute. Pour off bleach solution, replace with sterile water and allow the carrot to soak for at least one minute. Remove and dry with paper towels.

3. Using a sterile knife, cut the carrot longitudinally. Use a sterile scalpel to cut out a well in the center of one carrot half, as shown in Figure 1.

4. Place the roots in the well, packing snugly, as shown in Figure 2. Put the two halves back together and fasten with a rubber band on each end. Place the carrot in a Petri plate or other shallow plastic container to keep carrot dry during moist incubation.



5. Create a moist chamber, as shown in Figure 3, to house the carrot bait. Incubate for 4 to 9 days.

6. Separate the carrot pieces and dusty grey growth will be seen on both halves

(Figure 4). If *T. basicola* is present, chlamydospores of the fungus will be visible, under magnification, on the uncut half (Figure 5)..

7. To increase certainty, make a mount for the compound microscope and look for the black

chlamydospores, which are diagnostic for *T. basicola*. (Figure 6).

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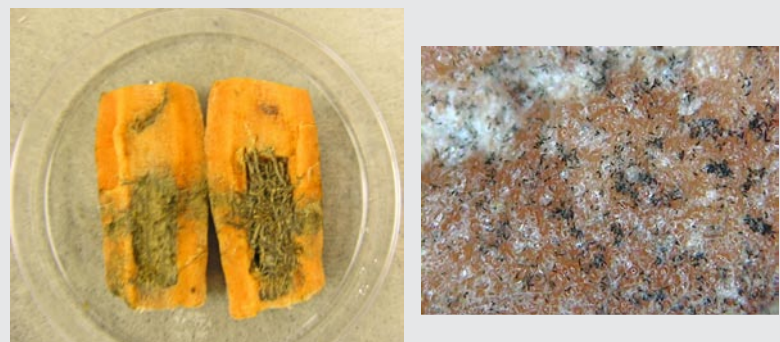


Figure 4 and 5. Bait showing growth of *T. basicola* (left) and, chlamydospores of *T. basicola* at 40x (right). Photos by Meg Williamson.

## Diagnostic Tip of the Month

# National Database

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8. You may also see the cylindrical conidia which are produced within conidiophores and emitted in chains (Figure 7).

## National Database Subcommittee Update

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The national database subcommittee met on December 12, 2007 to continue their work on reviewing the massive EPA pest and host lists and creating guidelines for uploading documents that will clarify how sample diagnoses should be transmitted to the national repository at Purdue

University. During this meeting a number of issues were addressed.

Please refer to the national database subcommittee web page of the [NPDN web site](#) for complete minutes of this meeting (login and password required).

Topics of discussion included:

- Change submission requests.
- Review of the 4th IT/Diagnosticians Meeting.
- Review of the bacteria list to discuss inconsistencies in common names.
- Review of the fungi codes common names beginning with the letter B.

The next meeting will be held on January 16, 2008.

## NPDN Information Security Awareness and Training Web Site



Mike Hill

Programmer Analyst  
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A new web site has been developed to provide online training for Information Security and Awareness. The web site can be accessed at <http://npdn-infosec.cerias.purdue.edu/>. There is a wealth of information available including several presentations describing operational security, system security, and physical security.

Please try out this new site and let us know what you think.

If you have difficulty using the site please contact [npdn-infosec@cerias.purdue.edu](mailto:npdn-infosec@cerias.purdue.edu) for assistance.

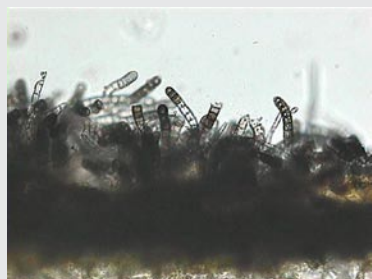


Figure 6 and 7. Chlamydospores of *T. basicola* at 200x (top) and conidia and conidiophores of *T. basicola* at 400x. Photos by Meg Williamson

