Diagnostics Subcommittee Update

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Subcommittee Chair
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The NPDN diagnostics subcommittee held a conference call on July 12, 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:

- Update on the subcommittee membership structure.
- 4th IT-Diagnosticians Meeting Plans- November 28-29, 2007.
- Reminder of SOP review to authors.
- Article on insect sample mailing procedures.
- Call for topics for the APS diagnostics subcommittee meeting.
- Request for information on obtaining positive controls.
- Announcement of the release of the NPDN Upload Guidelines version 1.0
- Reminder for SOP authors to consider submitting documents to PHP.

The next meeting will be held on **August 9, 2007**.

Diagnostic Tip of the Month: Techniques for Frequently Finicky Fungi Fond of Foiling Identification

Tom Creswell
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Figure 1. To avoid direct contact with water while still providing high humidity use a wire screen to lift incubated material off the wet paper towels. (Photo Tom Creswell, NCSU)

Some tissues rot quickly using the standard incubation technique of placing samples directly on wet paper towels. To avoid direct contact with water while still providing high humidity use a wire screen to lift incubated material off the wet paper towels (Figure 1). A cheap set of screens can be made by sawing off a 6 inch diameter PVC pipe into 1-2 inch segments, to which are glued circles of nylon window screening (Figure 2).

Incubating woody tissue for fruiting structures that never seem to appear:
Wisdom from Chuck Hodges, retired Forest Service Pathologist, who is the real brains behind ID of obscure fungi at the NCSU clinic, recommends this practice.

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Figure 2. A cheap set of screens can be made by sawing off a 6 inch diameter PVC pipe into 1-2 inch segments, to which are glued circles of nylon window screening. (Photo Tom Creswell, NCSU)

Diagnostic Tip of the Month

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If surface contaminants appear on woody stems during prolonged incubation then simply rinse them off, and put them back in the incubation chamber. It may be necessary to gently rub the stem or use a soft brush. The contaminants will come back, but in the meantime the pathogen may have had a chance to sporulate. Repeated application of this technique has worked with very stubborn fungi that take weeks to sporulate. Patience is a virtue here.

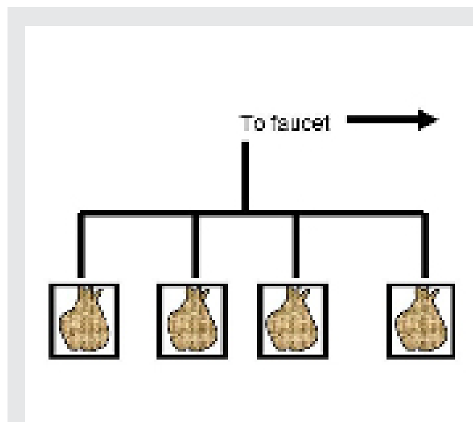

Figure 4. Richard Anacker's "In-the-sink" method for removing inhibitors to isolation. (Illustration by Richard Anacker, Maryland Department of Agriculture)

Figure 3. Incubate stems upright in large graduated cylinders with a small amount of water in the bottom. (Photo Tom Creswell, NCSU)

Richard H. Anacker, Plant Disease Specialist with the Maryland Department of Agriculture shares this technique for obtaining sporulation on woody tissues. Incubate stems upright in large graduated cylinders with a small amount of water in the bottom (Figure 3). Cover the top with Parafilm or foil.

This method works most of the time, even with canker fungi that only sporulate during a specific time of the season.

For those of you not in areas with water use restrictions; Rich also suggests prolonged washing (at least 1 to 2 hours) to remove inhibitors before isolating from roots. He has automated this process by building a manifold with stations for washing several root samples at once.

The diagram in Figure 4 illustrates his in-the-sink method which consists of tying roots in a cheesecloth bag, placing this in a beaker and directing a gentle stream of water into the beaker, letting the overflow run down the drain. About an hour of this washing, followed by letting the roots air dry slightly before plating, increases the percentage of successful root isolations when inhibitors are present.

For isolating fungi that form sclerotia in pure culture, Chuck Hodges uses a rapid technique that avoids any reduction in viability of the fungus. He cleans surface contaminants by placing a single sclerotium onto the surface of plain water agar. Under a dissecting scope, he rolls it around several times with a probe or forceps, then moves it to a new clean area of the plate and repeats the rolling 2-3 times. This has worked well with several species of sclerotia-forming fungi and is especially helpful for tiny sclerotia that may be killed by other means of surface decontamination.