NPDN National Plant Diagnostic Network

NPDN News

Volume 3 Issue 10, November 2008

National Updates

Select Agent Announcement- New Listing

Karen L. Snover-Clift, Cornell University

Advanced leaf blotching on soybean. Reproduced from G.L. Hartman (1987) Plant Disease 71:113-118.

The organisms on the Agricultural Bioterrorist Act of 2002 Select Agent listing have under gone a revision that became official on October 16, 2008. A proposed list had been distributed and was circulating for several months. The proposed list included the pathogens that cause Citrus Greening, *Candidatus* Liberobacter africanus and *Candidatus* Liberobacter americanus and *Phytophthora kernoviae* but these pathogens were not included in the final version. The revised list includes two new additions, red leaf blotch of soybean caused by *Phoma glycinicola* and gumming disease and ryegrass toxicity caused by *Rathayibacter toxicus*. Six other pathogens remained on the listing.

The complete listing can be found here and at the following website: http://www.apsnet.org/members/ppb/PDFs/SelectagentCFRNov2008.pdf

- ***Peronosclerospora philippinensis*** *(Peronosclerospora sacchari)***,** commonly known as Philippine downy mildew;
- ***Phoma glycinicola*** (formerly *Pyrenochaeta glycines)***,** red leaf blotch of soybean;
- ***Ralstonia solanacearum*****, race 3, biovar 2,** southern bacterial blight and brown root rot;
- ***Rathayibacter toxicus*****,** gumming disease and ryegrass toxicity;
- ***Sclerophthora rayssiae*** **var**. ***zeae***, brown stripe downy mildew;
- ***Synchytrium endobioticum*****,** potato wart;
- ***Xanthomonas oryzae*****,** rice bacterial leaf blight;
- ***Xylella fastidiosa*****,** citrus variegated chlorosis strain

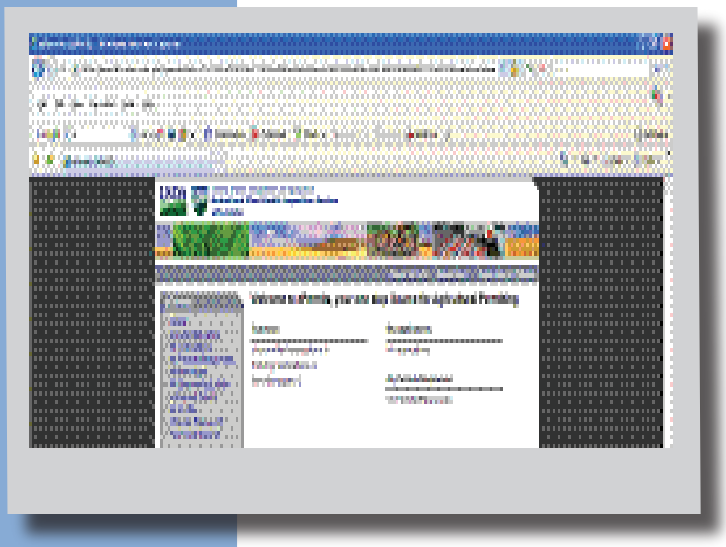
Issue Highlights:

National Updates

Applying for Diagnostic Permits to Receive Unknown Samples (USDA-APHIS-PPQ Form 526)

Karen L. Snover-Clift
Cornell University

Shortly after the network was created, the diagnosticians were tasked with acquiring permits from USDA-APHIS-PPQ to allow them to receive unknown samples from out of state. Our members were considered quite unique because our laboratories receive a wide variety of plant material with the possibility of numerous pests and pathogens being present versus the usual USDA-APHIS-PPQ permit requests that provide the host and pest/pathogen to be received. USDA-APHIS-PPQ and NPDN staff worked together to create a method for



the smooth submission and approval of permit requests. The NPDN diagnostics subcommittee provided a template for our members to follow which made the task very easy to perform and provided the USDA-APHIS-PPQ staff with all the information they needed to approve the requests.

Well, it has been a few years and the renewal process has begun for many laboratories. We were informed that to renew our permits, the renewal applications would have to be submitted using the on-line "ePermits" website. In April of 2008, Jan Byrne of Michigan State University suggested we again provide a tool that would make this process easier for our members. Jan's instructional article is located on the NPDN, Diagnostics Subcommittee webpage, in the "File Cabinet", http://www.npdn.org/Library/ViewDocument.pdf?filetype=pdf&DocumentId=8810. The document should be considered a guide, individual lab situations may vary requiring some modifications. Before you can begin the process, you must first create a user ID and password. The ePermit system performs various functions with different levels of authenticity required. To apply for permits you need level 2 authentication which requires that you visit a USDA Farm Service Agency and show a picture ID to verify your identity. Your account will then be give level 2 status.

If you have not submitted your application but plan to in the future, take a look at this step-by-step article. This article will be a big time saver for you!

Diagnostics Subcommittee Update

Karen L. Snover-Clift
Committee Chair
Cornell University
Department of Plant Pathology and Plant-Microbe Biology

The Diagnostics Subcommittee held a conference call on November 6, 2008. During this meeting a number of issues were addressed. Please refer to the website, http://www.npdn.org/DesktopDefault.aspx?tabindex=1&tabid=19, for complete minutes of this meeting.

- Review Potato Cyst Nematode, Beltsville- NPDN Diagnostician Training
- IT/Diagnosticians Meeting Agenda Discussion
- Diagnostician Survey
- Update on SOPs and new select agents
- National Meeting Update
- Bee Colony Decline

The next conference call will be held on Thursday, December 18, 2008.

National Database Subcommittee Update

Karen L. Snover-Clift
Committee Chair
Cornell University
Department of Plant Pathology and Plant-Microbe Biology

The National Database Subcommittee met on November 4, 2008 to continue our work on reviewing the massive EPA Pest and Host lists and revising 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 website, http://www.npdn.org/DesktopDefault.aspx?tabindex=1&tabid=40, for complete minutes of this meeting.

- Discussed change submission requests
- Discussed the IT/Diagnosticians meeting agenda
- Reviewed fungal disease common names beginning with the letter O-P.

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

Diagnostic Tip of the Month

Baiting Soil Using Leaf Pieces to Detect *Phytophthora* Species

Meg Williamson
Clemson University Plant Problem Clinic and Steve Jeffers, Clemson University Department of Entomology, Soils and Plant Sciences

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

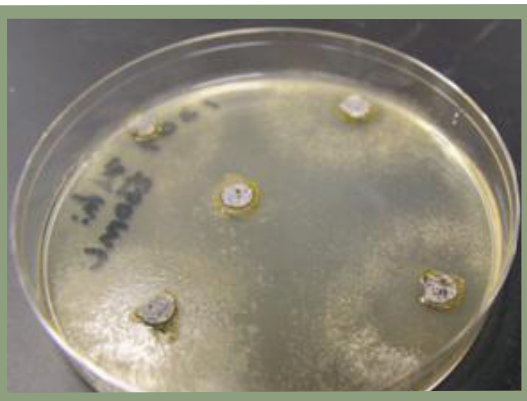


Figure 2

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.

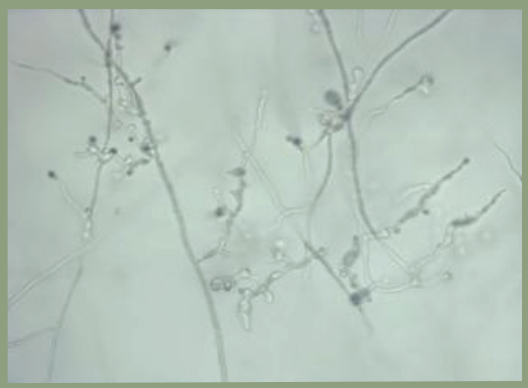


Figure 3

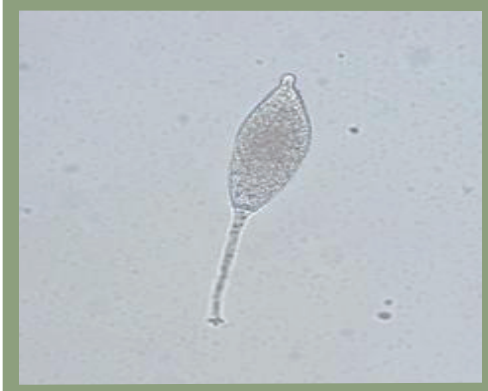


Figure 4

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.

IT Tip of the Month

NPDN Information Security Awareness and Training

CERIAS
Purdue University

It's Up to You!
Protecting sensitive and mission critical information is the job of everyone associated with the NPDN. The training provided on this site http://npdn-infosec.cerias.purdue.edu/ will help you. It is essential that you understand security issues and take steps to protect the information that the NPDN needs to accomplish its mission.

The Training Path
This site is designed to allow you to skip around to the information you need. Feel free to explore the content provided in any order. If this all seems overwhelming, we suggest the following learning path:

1. Introduction
2. Operational Security
3. System Security
4. Physical Security

We're Here to Help
If you have difficulty with this site, or need a question answered, please contact us. We can be reached at npdn-infosec@cerias.purdue.edu.

IT/Diagnostician Meeting Update

The 5th annual IT/Diagnosticians meeting was held in Chandler, Arizona on November 18-19, 2008. The focus of the meeting was on the lab management systems and developing a detailed strategic plan. Individual breakout sessions on lab systems were held on PDIS, DDDI, and the NC State system with a follow up summary presentation before the whole group. Updates on ipmPIPE, Phase 2, confidence levels, the data sharing policy, and Epidemiology were presented. From the strategic planning discussion it was determined that the priorities of the IT infrastructure activities were:

- Operate and maintain good security practices
- Operate and maintain regional and national databases
- Operate and maintain training and education web site.
- Improve record quality (more optional fields populated)
- Maintain and operate Exercise Scenario Module
- Operate and maintain national and regional web sites.
- Improve automation of QA/QC
- Evaluate, obtain, deploy, and test Secure Communications.

Complete details of the meeting with minutes and Powerpoint presentations will be posted on the web site soon.

Chandler, AZ

Workshop Opportunity

Past training participants.
photo: Paul Vincelli

A Real-time PCR workshop for applied plant pathologists will be held at the University of Kentucky on January 20-22, 2009. We have space available for two additional participants. Participants will design, execute, and interpret four real-time PCR experiments; extract DNA; and run an agarose gel. Topics include theory of real-time PCR, experimental controls, PCR inhibition, use of PCR kits, verifying amplicon identity, licensing, minimizing sample contamination, and troubleshooting. Primer design is not included in the workshop. For more information, contact Paul Vincelli (pvincell@uky.edu)

Exercise Updates

The NPDN chain of custody and chain of communication SOP that we use in our exercises and in real events has been revised. Please note that there are significant changes in the way to submit a sample to NIS or SEL for confirmation (step 2). There also are significant changes in notification procedures from APHIS. There will be improvements to the diagram coming soon, but in the mean time we wanted to get this out to you. Please familiarize yourself with this document before a real event comes up. Please distribute this to all NPDN diagnosticians. The full document can be found at http://www.npdn.org/Library/ViewDocument.doc?filetype=doc&DocumentId=10310

Laboratory Accreditation

2009 Proficiency Test Panel Announcement

Patrick J. Shiel
USDA-APHIS-PPQ-CHPST

The proficiency test (PT) panel for the 2009 *P. ramorum*, National Plant Protection Laboratory Accreditation Program (NPPLAP) is being prepared and will be distributed from December 2008 through March 2009.

The year 2008 marked the completion of four full years of inspections, training and proficiency testing in support of PPQ *P. ramorum* diagnostics. Like the 2008 panel, the 2009 *P. ramorum* PT panel is in only one format with two types of samples: DNA samples requiring PCR analysis and lyophilized tissue samples requiring DNA extraction and PCR analysis. Because the tissue samples require USDA permits to receive out-of-state diagnostic permits, please make sure that you have these permits up-to-date. Information and applications for permits can be viewed at the following USDA-APHIS-PPQ website: http://www.aphis.usda.gov/plant_health/permits/organism/index.shtml.

As with last year's panel, the lyophilized tissue samples were not designed to be tested by the approved ELISA test and are only provided to measure proficiency of the critical DNA extraction step in the *P. ramorum* diagnostic process. *P. ramorum* positive and healthy DNA controls for PCR will be provided for use with each panel and includes the second round nested PCR positive control.

To ensure the PT panels are of sufficient quality prior to shipment, the samples have been extensively tested using both Real-time PCR and conventional PCR according to USDA-approved protocols. Individual, randomly selected aliquots of each DNA panel samples are tested periodically to ensure DNA stability. DNA is also extracted from randomly selected lyophilized tissue samples at regular time intervals and tested by Real-time PCR to ensure tissue stability and uniformity.

In a continuing effort to be responsive to your requests a new diagnostic option for participants in the program will be available in 2009. A new real-time PCR based on Elicitin loci of *P. ramorum* was approved this year in addition to the ITS real-time PCR already in use. The two Real-time PCR assays will increase laboratory capacity and should decrease the potential of cross-reaction. Using these two USDA-validated Real-time PCRs can render a valid diagnostic determination for most *P. ramorum* samples only when used concurrently. The ITS-based Real-time PCR, which is the same assay used previously for diagnosis of *P. ramorum*, is combined with a Real-time PCR targeting the Elicitin loci in *P. ramorum*, with both assays multiplexed using different internal control reactions so that a complete diagnostic determination can be made.

Real-time PCR equipment
photo: Karen Snover-Clift

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Please note that CPHST has documented that performance of the combined Real-time PCR assay is not as sensitive for diagnosis as the previously developed conventional PCR assay, therefore inconclusive determinations will need to be resolved using the conventional (nested & multiplex) PCR. Although this can be accomplished by either demonstrated proficiency in the conventional PCR for 2009 or by forwarding DNA samples of inconclusive determinations to the USDA confirmatory lab, it is highly recommended that key coordinating diagnostic laboratories and other labs processing large numbers of samples in the network demonstrate and maintain proficiency in the conventional PCR to maintain national capacity for sample determinations. Demonstrated proficiency in the conventional PCR assay can resolve many inconclusive determinations and serve as a back-up system if Real-time PCR equipment should fail or need repairs during the tested season. The PT panels for certification of both the dual Real-Time PCR diagnostic and the conventional PCR are available to any lab currently participating in the USDA *P. ramorum* NPPLAP process.

Laboratories that wish to continue with the diagnostics from 2008 will have the two original options for certification available for 2009. This includes the use of the ITS Real-time PCR when used in a combination with conventional PCR, and the conventional PCR alone to provide diagnostic determinations.

Documents and diagnostic Work Instructions used for the Provisional Approval program can be downloaded in a PDF format at: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml

For the method(s) you intend to use, it is suggested that you order primers/probes/reagents (see tables below) in advance so that all required reagents are on hand before you receive your panel(s). Please be aware that fluorescent probes can take a minimum of 14 days to receive. For those who already have been certified in 2008, it is strongly suggested that you reorder a new set of primers (and probes), since degradation is one of the major reasons for failure or low efficiency of PCR. The DNA sequences of the primers and probes you will need to order to analyze the 2009 PT panel are listed on tables at the bottom of this letter. In addition, laboratories with DNA extraction kits that are expired are also urged to purchase new kits since expired kit components contribute to low DNA extraction efficiencies, often resulting in out-of-range Real-time PCR values and faint or missing conventional PCR product bands.

National Plant Protection Laboratory Accreditation Program
2009 *P. ramorum* Proficiency Test Panel

Lab name:
Fed-Ex information, including phone number:

Name of Diagnostician Requesting an SOD PT Panel	Type of Provisional Approval Sought ("C" for Conv PCR) ("R" for Conv & ITS Real-Time PCR) ("D" for Dual Real-Time PCR) ("B" for Dual and Conv PCR)	Send PT Panel (Indicate Month) (Dec. Jan., Feb., Mar)

Please complete the provided form and e-mail it to PPQ.NPPLAP@aphis.usda.gov

Page 1 of 1

Please Note: You will be provided with

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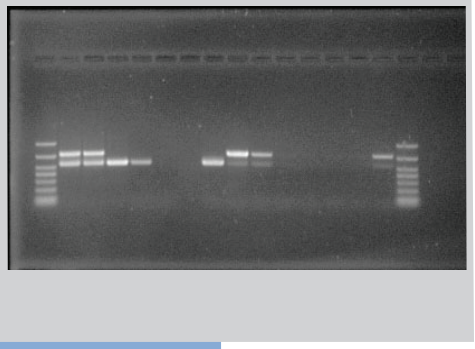
PT Work Instructions with the panel. These Work Instructions use the same probes/ primers, reagents and procedure as the sample diagnostic Work Instructions, but have additional guidelines for specifically handling and processing of the PT panel samples.



For 2009, you will be provided an example of the required format for the return of your proficiency test results. Returning your panel results in an unacceptable format will result in the return of your results for reformatting and resubmission, thereby delaying your evaluation. Upon receiving your properly submitted results, you will receive quick evaluation and prompt feedback.

Each individual participant should choose a preferred month to receive a panel(s) using the attached form and promptly return to the email address listed. Every effort will be made to accommodate your request(s). If you or your lab diagnostician is/are new to the program, please contact the NPPLAP program to complete required documentation to participate in the PT panel for 2009.

2009 National Meeting Update

Notice of Date Change
The NPDN National Meeting Associated Programs Committee members are developing exciting tours for the day before and the day after the National Meeting. The three tours in development are a Greenhouse Tour, a Production Agriculture Tour and a Botanical Garden/ Everglades tour. The Greenhouse tour will be offered on Sunday, December 6, 2008. The Production Agriculture tour has been moved from the 6th to Thursday, December 10, 2008 with the Botanical Garden/Everglades tour. For more information, please go to: http://www.npdn.org/DesktopDefault.aspx?tabindex=7&tabid=70

Miami, FL
photo: Karen Snover-Clift

Upcoming Events

National Events

March 24-26, 2009, Sixth International IPM Symposium, Portland, OR
December 6-10, 2009, NPDN National Meeting, Miami, FL

Diagnostician Training Events

Citrus Greening, USDA-APHIS-PPQ-CPHST-NGBTL, Beltsville, Md.
February 3, 2009

Phytophthora kernoviae, USDA-APHIS-PPQ-CPHST-NGBTL, Beltsville, Md.
February 10-12, 2009
February 17-19, 2009
March 3-5, 2009
March 10-12, 2009

Potato Wart, USDA-APHIS-PPQ-CPHST-NGBTL, Beltsville, Md.
March 17-19, 2009
April 7-9, 2009
April 17-19, 2009

Regional Events

January 21-22, 2009, GPDN Annual Meeting, Fargo, ND
March 18-19, 2009, NEPDN Annual Meeting, New Brunswick, NJ

Editing by
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