

Operations Committee Meeting in Davis, CA

Richard Bostock, NPDN Executive Director, University of California at Davis, Department of Plant Pathology


The NPDN Operations Committee convened at the Department of Plant Pathology at the University of California, Davis, on May 11-13, 2010. The agenda included review of the NPDN strategic plan, discussion with National Plant Board leaders, program committee updates, laboratory accreditation overview, and a brainstorming session on diagnostic research needs and how the NPDN might facilitate and participate in related initiatives. The operations committee meeting coincided with the annual meeting of the National Clean Plant Network (NCPN), which also was held on the Davis campus. This provided an opportunity for both groups to interact and to discuss areas of mutual interest, particularly in the delivery of training and outreach programs. The NCPN also graciously hosted the operations committee at a banquet where additional informal interaction took place.

NPDN participants included regional directors and associate directors, regional center staff and at large representatives, members of the Center for Environmental and Regulatory Information Systems (CERIS, Purdue), and representatives from the USDA-NIFA, ARS, APHIS, and the California Department of Food and Agriculture

(CDFA). Participants also enjoyed the sights, sounds, and tastes of the nationally acclaimed Davis Farmers' Market, and tours of the UC Davis Foundation Plant Services and the CDFA



Operations Committee Meeting, Davis, CA. Photo courtesy of Dick Hoenisch.

Plant Pest Diagnostics Laboratory in Sacramento. The meeting was planned and hosted by the WPDN Regional Center at UC Davis. 

Issue Highlights:

- Tip - Immunosorbent Electron Microscopy
- TCD Workshop
- Bioinformatics Workshop
- NPPLAP Accredited Labs
- PDIS 2.0 Diagnosticians' Survey Available
- IT Security Tip - Phishing



National Institute of
Food and Agriculture

Diagnostic Updates

Immunosorbent Electron Microscopy (ISEM) Using Crude Plant Extract for

Detection of Plant Viruses

Dimitre Mollov and Ben Lockhart, University of Minnesota, Department of Plant Pathology

The University of Minnesota Plant Virology Laboratory and Plant Disease Clinic would normally utilize ISEM using partially purified extract versus the described crude extract below. The crude extract can be used in place of partially purified virus extract when diagnosticians do not have access to an ultracentrifuge.

1. Sample preparation: Grind 0.1 g tissue (using mortar and pestle or sample mesh bag) in 1-2 ml phosphate buffer (100 mM, pH 7.0). Mix 500 μ l sample extract and 500 μ l CHCl_3 in 1.5 ml tube and vortex vigorously. Centrifuge for 5 minutes at full speed. Use supernatant for examination.
2. Coating: Dilute whole antiserum (AS) 1/500 in ELISA carbonate coating buffer; Incubate TEM grid, film side down on 10 μ l diluted AS in a moist chamber for 15-20 minutes.
3. Rinsing: Transfer the grid briefly to 10 μ l 100 mM phosphate buffer pH 7.0.
4. Trapping: Transfer the AS coated grid to 10 μ l clarified sample extract. Incubate 15 minutes (at room temperature) to overnight (at 4°C). For low virus concentration shake gently in platform shaker at room temperature.

5. Decoration: Transfer grid without rinsing to 10 μ l AS diluted 1/250 in PBS.
6. Staining for TEM: Rinse grid with 4-6 drops (10 μ l) Phosphotungstic acid - 2% (PTA) NaOH pH 7.0 or 4-6 drops dH_2O and 2 drops Uranyl acetate - 1% (UA).
7. Examination: Use the grid for TEM.



Bean Yellow Mosaic Virus, decorated with antibody.

Note that even though there are broken pieces the decoration indicates that they are BYMV. Photo courtesy of Dimitre Mollov.

Phosphate Buffer:

100 mM NaPO_4 (mono and dibasic) pH 7.0

Carbonate Coating Buffer:

60 mM $(\text{Na})_2\text{CO}_3$ pH 9.6. 🌱

Black Walnut Thousand Cankers Disease Workshop

Ned Tisserat, University of Colorado,
Department of Bioagricultural Sciences
and Pest Management

A workshop on thousand cankers disease (TCD) of walnut is scheduled for June 23rd in Golden, Colorado (near Denver). The purpose of the workshop is to show participants symptoms and signs of TCD in the Denver Metro area and to provide up-to-date information on detection, diagnostics and surveillance methods for this new disease. This workshop is intended for researchers/extension personnel, diagnosticians, regulatory officials and other professionals

Transportation from designated hotels to the workshop will be provided.

The number of out-of-state participants is relatively small so far, so I have *not* reserved a block of rooms at any motel. Instead, I suggest that out-of-state travelers choose from one of the motels listed below. Please let me know which hotel you chose so that we can pick you up the morning of the workshop.

Thousand Cankers Disease Workshop:

June 23, 2010

Jefferson County Fairgrounds
Golden, CO

Register TODAY!

Tentative Schedule (8:30 AM – 5:00 PM)

8:30 AM	Refreshments and introductions
8:45 AM	Overview of thousand cankers disease (TCD)
10:00 AM	Update on TCD quarantines or other regulatory issues
10:45 AM	Lab workshop on identification of <i>Pityophthorus juglandis</i> and <i>Geosmithia</i> spp. <ul style="list-style-type: none">• Morphological features of <i>Pityophthorus</i>, other beetles associated with TCD<ul style="list-style-type: none">○ Other arthropod topics (trapping, attractants)• Morphological characteristics of <i>Geosmithia</i><ul style="list-style-type: none">○ Distinguishing features of <i>Geosmithia</i>, growth, etc.○ Other fungi associated with TCD (<i>Fusarium</i> spp., others)○ Other <i>Geosmithia</i> species○ Isolation and maintenance techniques
12:00 PM	Catered lunch
12:45 PM	Dissection of felled trees and other demonstrations at fairgrounds
2:30 PM	Bus tour to view symptoms of TCD trees in Denver

interested in walnut production or preservation. The workshop is sponsored by Colorado State University Extension, NPDN, the Colorado Department of Agriculture, Denver, Boulder and Wheat Ridge Parks and Recreation, USDA Forest Service, and USDA-NIFA.

Those interested in attending the workshop should contact Ned Tisserat (ned.tisserat@colostate.edu). Please indicate the number of people from your institution who would like to attend and the method of arrival (by air to DIA or car). Participants must provide their own transportation and lodging.


- Hampton Inn – Denver International Airport (DIA)
- La Quinta Inn – DIA
- Hyatt Place Denver Airport – Aurora, CO
- Crown Plaza – DIA
- Ramada Inn and Suites – DIA

Those driving to Colorado can choose their own hotels and then meet the group at the Jefferson County Fairgrounds in Golden. 🌿

NPDN-USDA APHIS 2010 Now Offering Advanced Diagnostic Training on Bioinformatics

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

Laurene Levy, USDA-APHIS-PPQ-
CHPST-NPGBL



The NPDN Diagnostics Program Area Committee and members of USDA-APHIS-PPQ-CHPST-National Plant Germplasm and Biotechnology Laboratory (NPGBL) are offering training sessions on Bioinformatics this summer. The sessions are offered the weeks of July 12, 2010 (July 12, 13, and 14 until noon, July 14 starting at 1 pm, 15, and 16) and August 23, 2010. (August 23, 24 and 25 until noon, August 25 starting at 1pm, 26 and 27). The four 2 ½ -day sessions will cover analysis of obtained sequences from both plus and minus strands, editing sequences, blasting sequences, understanding blast results based on size and gene target, when to directly sequence PCR products or clones, which genes are used for sequence analysis for fungi, bacteria, and viruses, what sequence analysis programs are available commercially or as freeware, and hands-on use of sequence analysis programs using sequences from case studies for different pathogen types. Participants of this meeting are expected to cover their travel, lodging and meal expenses. There is no registration charge for the meeting or for meeting materials. These expenses are covered by our colleagues at USDA-APHIS-PPQ-CPHST-NGBTL. If you are interested in participating in any of these workshops please contact Karen Snover-Clift at kls13@cornell.edu. 

National Plant Pathogen Laboratory Accreditation Program (NPPLAP) Approved Labs for 2010

The National Plant Protection Laboratory Accreditation Program (NPPLAP) evaluates laboratories using molecular diagnostics for APHIS-PPQ. NPPLAP currently accredits laboratories to diagnose *Phytophthora ramorum* and the Huanglongbing (citrus greening) pathogen. In order to become accredited, labs need to first pass an inspection and then pass a blind Proficiency Test Panel.

NPPLAP Documents

Please contact Dr. Pat Shiel at if you have questions or plan to participate in NPPLAP either by establishing an accredited laboratory in this system or becoming a certified analyst.

- Introduction to *P. ramorum* Program
- Proficiency Test Letter for *P. ramorum*
- *P. ramorum* Lab Guide
- *P. ramorum* Checklist
- Potentially Actionable Suspect Sample Policy for *P. ramorum*
- Approved Labs for *P. ramorum* Testing 
- Proficiency Test Letter for Huanglongbing
- Huanglongbing Checklist
- Approved Labs for Huanglongbing Testing 

Up-to-date lists of the approved diagnostic labs can be found at www.aphis.usda.gov/plant_health/cphst/npplap.shtml. 



New Online Diagnostic Resources

A series of Canada/Alaska spider handbooks and a wide variety of other entomological monographs are now available online (PDF) through the Entomological Society of Canada website at: www.esc-sec.ca/aafcmono.html

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E**Diagnostics Committee**

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

Since the last newsletter, the Diagnostics Committee held a conference call on May 6, 2010. During this meeting, a number of issues were addressed. Please refer to the website, www.npdn.org/diagnostics, for complete minutes of this meeting (login and password required).

- Basic technique workshop update
- SOP updates
- Chain of Communications SOP
- Gap-Surge capacity analysis
- Beltsville trainings
- New members to the committee
- List of NPPLAP approved laboratories on diagnostics webpage

The next conference call will be held on Thursday, June 10, 2010.

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E**Exercise Subcommittee**

Sharon Dobesh, Committee Chair
Kansas State University, Department of Plant
Pathology

The Exercise Committee conducted a conference call on Friday, May 21, 2010. The following agenda items were discussed:

- Update on Chain of Communications document (merged Diagnostic and Exercise SOPs)

- Update on Mike Stubb's position
- APHIS exercises
- Scheduled NPDN exercises
- Other business

The next conference call is scheduled for Tuesday, June 15, 2:00 PM EST.

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E**National Database Subcommittee**

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

Since the last newsletter, the National Database Committee held a conference call on May 5, 2010. The committee continues to work on reviewing the massive NPDN 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 National Database page on the website, www.npdn.org (login and password required), for complete minutes of this meeting.

- Discussion of change submissions
- Discussion of fungi pest codes beginning with scientific names Q, and R

The next meeting will be held on June 9, 2010.

IT Subcommittee

Eileen Luke, Committee Chair
Purdue University, Center for Environmental and
Regulatory Information Systems

The IT Subcommittee held a conference call on
May 4, 2010. The committee discussed:

- NPDN IT/Diagnosticians meeting - discuss
conflicts with October dates
- Discuss draft of IT Program Committee
presentation for upcoming Ops Comm meeting
in Davis, CA

If you would like to read the complete minutes from
this meeting visit the IT webpage on the NPDN
website at www.npdn.org (login and password
required).

The next conference call is scheduled for Tuesday,
June 8, 2010 at 11:00 AM EST.

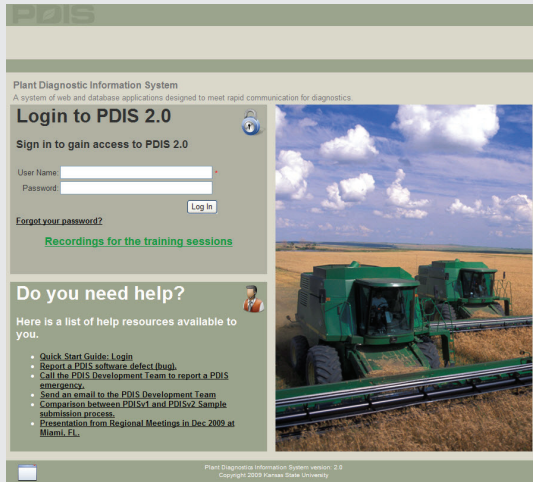
Visit the NPDN homepage at www.npdn.org for more information on specific Program Area Committees.

Announcements ~ Membership information ~ Committee reports and meeting minutes ~ Documents and SOPs

Diagnosticians' Survey for PDIS 2.0

Jan Byrne, Michigan State University and
Karen Scott, Cornell University

[PDIS Users, we need your input!](#)



Significant efforts were put into the redesigning and updating of PDIS to create PDIS 2. The diagnostician model of PDIS 2. has been in use for several months now. An online survey specific to the diagnostician module has been created to capture users' opinions and comments on their experiences with PDIS 2. The survey is intended to be completed by diagnosticians and personnel with the sample check in role. Information captured by the survey will be used to guide future changes/enhancements to PDIS 2. Your answers to the survey will be anonymous, although your name and contact information can be entered, Survey Monkey does not associate it with your responses.

Your input is VERY important for the programmers to be able to make changes and enhancements to the program to better serve the users. The survey has 29 questions so please allow yourself the time needed to provide thoughtful responses. Please complete the survey by June 11, 2010. Thank you in advance for your time and input. [Click here to complete the survey.](#) 🌱

Security Tip of the Month: Phishing Attacks

Michael Hill, Purdue University, CERIS

Phishing is a term used to describe a type of attack where users are lured, typically via e-mail, into clicking on a malicious link. This link then redirects them to a legitimate looking website that attempts to capture sensitive information. As the Internet has evolved and the number of phishing targets has increased, these attacks have become more difficult to detect.

A good security practice to follow is to never click on a link in an e-mail. Instead, manually type the link into your browser's address bar so that any malicious code embedded in the link will not be executed. If you go to a link and a site asks for any information you consider sensitive, verify through separate channels (for example, via a phone call) that a site is legitimate.

SonicWall has created a quiz which provides good examples of phishing attacks. I encourage you to take the quiz available at www.sonicwall.com/phishing and see how well you can detect phishing attacks. 🌱

IT News

IT/Diagnosticians' Meeting

Keep the dates of Wednesday October 13th and Thursday October 14th open for the next IT/Diagnosticians' meeting to be held in Chandler, AZ. Please do not make any travel arrangements at this time, until we have sent out the final notice. We are currently exploring the possibility of combining this meeting with an Operations Committee meeting. More information will follow in next month's newsletter.



Web Portal Updates

Karen Scott, Cornell University, Department of Plant Pathology and Plant-Microbe Biology

The NPDN National web page has a new look. Rotating images have been added to the banner at the top of each page. Additional new features are being developed and will be deployed when they are completed. Access to the regional sites has been given to the appropriate developers in their respective regions and will be brought on line as soon as they are ready. 🌿

Regional Updates



Emerald Ash Borer Found in Iowa

On May 14, 2010 the Iowa Emerald Ash Borer Team confirmed that the emerald ash borer has been found in Iowa along the Mississippi River on land owned and managed by the U.S. Army Corps of Engineers and the U.S. Fish and Wildlife Service. An infestation in nearby Victory, Wisconsin was discovered in 2009.



Dorsal view of emerald ash borer.
Photo by Kent Loeffler.

[Click here](#) to read the full announcement from the Iowa Department of Agriculture and Land Stewardship. 🌿

Upcoming Events

National Events

June 23, 2010
Black Walnut Thousand
Cankers Workshop
Golden, CO

July 14-15, 2010
Tree Pests of the Great Plains
Chadron, NE

August 7-11, 2010
APS Annual Meeting
Charlotte, NC

September 20-24, 2010
17th Ornamental Workshop on Diseases
and Insects
Hendersonville, NC

December 12-15, 2010
ESA Annual Meeting
San Diego, CA

November 6-8, 2011
NPDN National Meeting
San Francisco, CA