

## National Updates

### Getting the Most Out of the NPDN Web Site: Where are the SOPs?

The NPDN continues to populate [www.npdn.org](http://www.npdn.org) with training, diagnostic, and administrative information and documents. We think it might be a good time to start updating all members on the contents of the web site to ensure users can find what they need. This month we'll direct you to the NPDN diagnostic SOPs.

The NPDN Diagnostic SOPs are written by NPDN diagnostic personnel, with assistance from subject matter experts

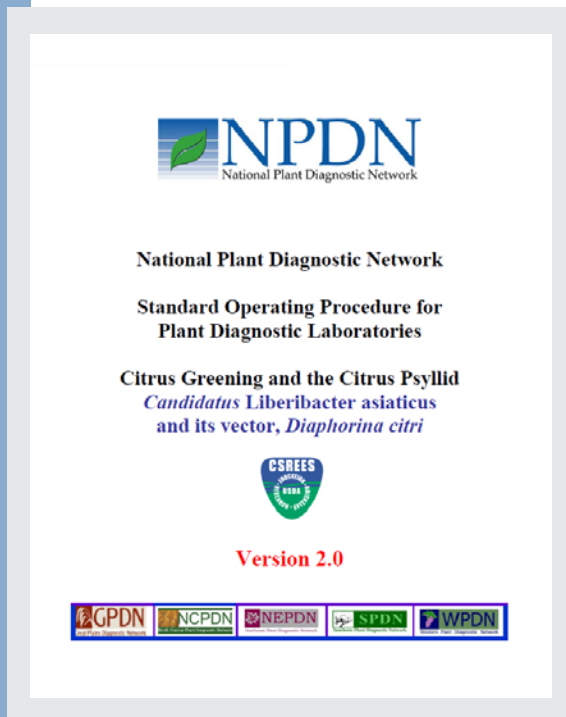
from research, extension, and regulatory institutions, and managed by the NPDN diagnostics subcommittee.

Several new SOPs have been posted to the NPDN web site over the past few months, and many more have been updated in the past year. Posting new versions to the web site allows all the most up-to-date information to be housed in one place for your convenience.

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#### **Issue Highlights:**

- ◆ Getting the Most Out of the NPDN Web Site: Where are the SOPs?
- ◆ Diagnostics Subcommittee Update
- ◆ Employment Opportunity
- ◆ Diagnostic Tip of the Month: Preparation and Storage of Primers and Probes
- ◆ National Database Subcommittee Update
- ◆ National Database Updates: Mark Your Calendars: IT/Diagnosticians' Meeting and IT Tip of the Month: Lock that Computer
- ◆ 2009 North Central APS Division Meeting Announcement
- ◆ NEPDN 2009 Advanced First Detector Webinars



# National Updates

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You can find the NPDN SOPs by first logging into the NPDN web site ([www.npdn.org](http://www.npdn.org)) with your

NPDN/PDIS credentials, then navigating to the column of options on the left-hand side of the page. Click on Committees, then Diagnostics. Once on the Diagnostic Committee page, scroll down (about ¼ of the way down the page) until you see the heading for the SOPs: “Pathogen/Pest Standard Operating Procedures”.

Other documents contained in this section include the NPDN Exercise Chain of Custody SOP, a PPV training manual, and for those participating in the USDA laboratory certification, panel worksheets.

Also on the web site is a section that details the changes made to current SOPs. This section is located directly below the section of SOPs and was added to help our users with updates. It will be labeled with the heading: “Updates to SOPs.” Some updates are minor and if you keep hard copies, you may only need to print off a few pages to update your SOP with the most current information. Others may require a complete re-print. This section will tell you exactly what changes have been made to the SOPs.

The diagnostics subcommittee members are currently working on creating new SOPs for the organisms that were added to the USDA select agent listing in the Federal Register on October 16, 2008.

<a href="#">NPDN Chain of Custody SOP 10-13-08</a>
<a href="#">Agricultural Bioterrorism Agent and Toxin List</a>
<a href="#">Brown Stripe Downy Mildew Version 2.0</a>
<a href="#">PHP Article: Brown Stripe Downy Mildew (<i>Sclerophthora rayssiae</i> var. <i>zeae</i>) of Maize</a>
<a href="#">Citrus Greening Version 2.0</a>
<a href="#">Laurel Wilt version 1.0</a>
<a href="#">Phakopsora pachyrhizi Version 2.2</a>
<a href="#">Phytophthora ramorum Version 1.1</a>
<a href="#">Pink Hibiscus Mealybug draft Version 1.1</a>
<a href="#">Plum pox potyvirus Version 1.5</a>
<a href="#">Cornell University PPV Training Manual, Version 2.0</a>
<a href="#">Cornell University PPV Training Presentation, Version 2.0</a>
<a href="#">Ralstonia solanacearum R3 B2 Version 2.3</a>
<a href="#">Soybean Aphid Draft 1.5</a>
<a href="#">Synchytrium endobioticum Draft 1.0</a>
<a href="#">PANEL WORKSHEET- Multiplex</a>
<a href="#">PANEL WORKSHEET- Double Nested</a>
<a href="#">PANEL WORKSHEET- Real-time PCR</a>

The current list of pathogen/pest standard operating procedures on the NPDN web site.

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Karen Rane of the University of Maryland is working on the SOP that will be created for Red Leaf Blotch of Soybean caused by *Phoma glycinicola*. Melodie Putnam of Oregon State University has agreed to lead the development of the SOP for Gumming Disease and Ryegrass Toxicity caused by *Rathayibacter toxicus*.

*Phytophthora kernoviae* was considered for the new select agent list but was not included. Because of its importance, the diagnostics subcommittee members decided that it would be of value to create an SOP for this pathogen, therefore, Karen Snover-Clift will be working on this project.

Another pathogen of interest but not on the list is the White Potato Cyst nematode, *Globodera pallida*. Craig Webb, the Western Region Identifier for USDA-APHIS-PPQ, has agreed to create this SOP for the NPDN.

Having all these SOPs readily available for our NPDN members and collaborators will ensure the proper handling of suspect samples that enter the network laboratories for processing, which in turn, will ensure the rapid identification of hazardous pathogens and pests, therefore, reducing the impact of an introduction.

### **Diagnostics Subcommittee Update**

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

The NPDN diagnostics subcommittee held a conference call on May 14, 2009.

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# National Updates

Title	Date of Change	Vers. To Vers.	Pages to Update Manual
Soybean Rust ( <i>Phakopsora pachythizi</i> )	06/05/2007	Ver. 1.9 to Ver.2.0	2-36
Soybean Rust ( <i>Phakopsora pachythizi</i> )	04/07/2008	Ver. 2.0 to Ver. 2.1	8-9
Sudden Oak Death ( <i>Phytophthora ramorum</i> )	04/07/2008	Draft 5.5 to Ver. 1.0	All
Soybean Rust ( <i>Phakopsora pachythizi</i> )	04/15/2008	Ver. 2.1 to Ver. 2.2	17
Sudden Oak Death ( <i>Phytophthora ramorum</i> )	07/18/2008	Ver. 1.0 to Ver. 1.1	11, 16 and 44
Citrus Greening	02/10/2009	Ver. 1.0 to Ver. 2.0	All
Soybean Aphid	02/19/2009		All

Updates to NPDN Diagnostician Standard Operating Procedures.

# Diagnostic Updates

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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:

- Scheduling potato wart workshops, Beltsville-NPDN Diagnostician Training
- Basic technique workshop update
- Status of SOPs and new select agent SOPs
- Template for NPDN SOPs for plant diagnostic laboratories

The next conference call will be held on Thursday, June 11, 2009.

## California Department of Food and Agriculture Plant Pathology Diagnostician Position Available

Under the general supervision of the Branch Chief the incumbent will serve as an official scientific resource, providing timely and accurate plant disease diagnostics of the most critical and unusual plant diseases that may be actual or potential pests of California Agriculture, will provide professional expertise equivalent to the highest academia level of State, County and Federal Government and Scientific Colleagues throughout the world.

More information on this position is posted as [job listing 3357](#) on the APS placement web site.

## Diagnostic Tip of the Month: Preparation and Storage of Primers and Probes

Elizabeth Bush

Virginia Tech Plant Disease Clinic

Proper preparation and storage of primers and probes is critical for optimal PCR results. The procedure our lab uses for primer/probe preparation and storage consistently results in oligos that maintain optimal efficiency for at least two years in  $-80^{\circ}\text{C}$ , if not longer. Long-term stock solutions prepared in this manner should retain optimal viability for several years, as long as numerous freeze and thaw cycles are avoided. Avoid repeated freezing and thawing by preparing aliquots of working stock solution (typically at  $10\ \mu\text{M}$  concentration) adequate for approximately 3 months-worth of PCR assays.

### *Suspending Lyophilized Oligos at $100\ \mu\text{M}$ Stock Concentration for Long-term Storage*

Preheat water bath to  $50^{\circ}\text{C}$ . Wipe down work area and equipment with DNA-Erase or similar product. Spin down oligos briefly (30 seconds at 10,000 rpm) in microcentrifuge to ensure oligos are in the bottom of the tube. To prepare a  $100\ \mu\text{M}$  stock solution add an amount of 1X Tris-EDTA (TE), pH 8.0, equivalent to ten times the amount of nanomoles of oligos listed on the oligo spec sheet (e.g. for 35.2 nmoles add  $352\ \mu\text{l}$  1X TE). Vortex the stock solution for 30 seconds at 3,000 rpm. Place in  $50^{\circ}\text{C}$  water bath for 10 minutes.

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Vortex again for 30 seconds at 3,000 rpm and place in water bath for 10 minutes. Use this procedure to prepare working stock solution aliquots, then seal with Parafilm and store at -80°C.

### Preparing Working Stock Solution (typically 10 μM) Aliquots

Although DNA is not as stable in molecular grade water as in TE, we prefer to prepare our primer/probe working stock solutions in molecular biology grade water (MBW). This is because EDTA, a component of TE, may inhibit PCR.

Label microfuge tubes for working stock solutions appropriately. UV-disinfect the open microfuge tubes in a UV-light equipped laminar flow hood, if available, for 15 minutes. Dispense 90 μl MBW to each tube. Add 10 μl 100 μM stock solution to each tube. Vortex working stock solutions for 30 seconds at 3,000 rpm. Seal tubes with Parafilm and place in -20° or -80°C freezer.

I typically keep tubes that are likely to be

used within the next month at -20°C and put the remainder in the -80° freezer to retrieve when needed.

## Diagnostic Tip of the Month



Figure 2. Fiberboard storage boxes with tube dividers can be numbered/lettered to correspond with primer/probe inventory entries in an Excel spreadsheet.

### Some Advice on -80°C Storage

Maintaining an Excel spreadsheet with information on primers/probes stored in the -80°C freezer is a worthwhile task that saves much time in the long run (Figure 1).

Fiberboard storage boxes and dividers (Fisher Scientific catalog #'s 11-678-24A and 13-989-218) can be numbered to correspond with spreadsheet entries (Figure 2).

Box Location	Box Number (1-9)	Box Location Letter (A-J)	Item Description	Date Stored	Concentration	Solution (e.g. IX)	Notes
3	1	A	ITS1Ranf4a	10/4/2007	100 uM	IX TE	phaktopora primer--single PCR Shors
4	1	A	ITS1Ranf4a	5/29/2008	10 uM	0.1X TE	phaktopora primer--single PCR Shors
5	1	A	Ppa2 primer	10/4/2007	100 uM	IX TE	phaktopora primer--Reid Frederick
6	1	A	CM45	3/31/2009	100uM	IX TE	Clavibacter michiganensis subsp. michiganensis
7	1	A	CM46	3/31/2009	100 uM	IX TE	Clavibacter michiganensis subsp. michiganensis
8	1	A	FGP56-03	3/31/2009	100 uM	IX TE	16S rRNA universal primer FOR
9	1	A	FGP1.132-38	3/31/2009	100 uM	IX TE	16S rRNA universal primer REV
10	1	A	ITS6_forward	7/14/2008	5 pmol/ul	NFW	phytophthora for sequencing
11	1	A	ITS6_forward	1/24/2008	100 uM	IX TE	phytophthora for sequencing
12	1	B	ITS4_reverse	1/24/2008	100 uM	IX TE	phytophthora for sequencing
13	1	B	COX-RW	1/31/2008	100 uM	IX TE	primer stock for p. ramorum real-time
14	1	B	COX-F	1/31/2008	100 uM	IX TE	primer stock for p. ramorum real-time
15	1	B	Primo-1527-190R	1/31/2008	100 uM	IX TE	primer stock for p. ramorum real-time
16	1	B	Primo-114F	1/31/2008	100 uM	IX TE	primer stock for p. ramorum real-time
17	1	B	Primo-1527-134T	1/31/2008	100 uM	IX TE	probe stock for p. ramorum real-time
18	1	B	COX-P	1/31/2008	100 uM	IX TE	probe stock for p. ramorum real-time
19	1	B	102U-F-259L-R primer mix	12/17/2008	4 uM	NFW	w.s. Ekinin real-time primer for P. ramorum
20	1	B	102U-F-259L-R primer mix	12/17/2008	4 uM	NFW	w.s. Ekinin real-time primer for P. ramorum
21	1	C	CM45	3/31/2009	10 uM	NFW	Clavibacter michiganensis subsp. michiganensis
22	1	C	CM45	3/31/2009	10 uM	NFW	Clavibacter michiganensis subsp. michiganensis
23	1	C	CM46	3/31/2009	10 uM	NFW	Clavibacter michiganensis subsp. michiganensis
24	1	C	CM46	3/31/2009	10 uM	NFW	Clavibacter michiganensis subsp. michiganensis

Figure 1. An inventory of primers/probes maintained at -80°C is maintained in an Excel spreadsheet.

# National Database

## National Database Subcommittee

### Update

Karen L. Snover-Clift  
Committee Chair  
Cornell University

## NPDN National Database

Michael Hill, CISSP  
NPDN Project Administrator /  
Programmer Analyst  
CERIS - Purdue University

Department of Plant  
Pathology and Plant-  
Microbe Biology

The NPDN national database subcommittee met on May 14, 2009 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.



Purdue University, site of the 2009 NPDN IT/Diagnostician's Meeting.

## IT/ Diagnosticians' Meeting

The 6th annual IT/Diagnosticians' meeting will be held at Purdue University on September 29-30, 2009. A block of rooms for the meeting are available at the University Plaza Hotel in West Lafayette, IN at a discounted rate of \$92 per room. Reservations can be made by calling (800) 777-9808 and mentioning

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 submissions
- disease synonyms
- assignment of new groups for review

The next meeting will be held on June 10, 2009.

“NPDN.” More information on the hotel can be found by visiting their website at <http://universityplazahotelwestlafayette-px.trvlclick.com/>. Please stay tuned to the newsletter in the coming months for more details.

## IT Security Tip of the Month: Lock that Computer

Do you lock your computer when you step away? An unlocked computer provides opportunities for someone to perform malicious actions such as sending e-mails, installing spyware, modifying or destroying files, and many other dangerous activities.

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It's important to lock your screen anytime you step away from your computer so that you can be protected against these types of attacks. Additionally, a screen saver that automatically locks the screen should be enabled.

If you are running Windows XP or Vista holding down the <Windows Key> + <L> key will quickly lock your screen. Older versions of Windows can also be locked by holding down the <Ctrl> + <Alt> + <Del> key combination.

It's easy to lock your screen and well worth the time it takes to unlock the screen when you return to your computer.

If you have any questions regarding how to lock your specific computer please contact your IT representative.



## *North Central Region*

### **APS 2009 North Central Division Meeting**

The 2009 APS North Central Division meeting will be held June 21-23, 2009 in Ames Iowa. Four workshops including a corn nematode workshop will be held in conjunction to the meeting.

Additionally, four key symposia are planned for the meeting including one on crop biosecurity.

For more information about these tours and the conference, please visit on the web: [2009 APS-NC Division Meeting](#).



## *Northeast Region*

### **NEPDN 2009 Advanced First Detector Webinars**

The NEPDN has hosted two advanced first detector webinars this spring with four total planned for spring/summer of 2009. The first webinar, "The Art and Science of Diagnosis: Insect Pests on Plants" was presented by Carolyn Klass, Senior Extension Associate, Department of Entomology at Cornell University on April 23, 2009 and was viewed by approximately 55 participants from all over the Northeast region.

The second presentation "Art and Science of Diagnosis: Plant Diseases" was presented by Karen Snover-Clift, NEPDN associate director and director of the Cornell Plant Disease Diagnostic Clinic on May 14, 2009 with over 40 participants from all 12 states in the Northeast region.

Two more webinars are planned for June and July. These will be presented by Dave Clement, Karen Rane and Mary Kay Malinoski of the Home and Garden Center at the University of Maryland and will focus on problems of woody ornamentals reflecting the samples that are currently being received by the diagnostic laboratory during those time periods.

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# Regional Updates

# Regional Updates

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Although the NEPDN regional center continues to learn the ins and outs of Adobe Connect, the presentations to date have been well received with only a few minor technological difficulties.

Lillian Dorchak, a master gardener in New Jersey, wrote, “Thanks so much for this opportunity, it is really valuable, especially at our Master Gardener Helpline here in Hunterdon County New Jersey.”



**Art & Science of  
Diagnosis:  
Plant Diseases**

First Detector Training  
May 14, 2009

Karen L. Snover-Clift  
Director, Plant Disease Diagnostic Clinic  
Associate Director, NEPDN  
Cornell University





## *National Events*

**August 1-5, 2009, [2009 APS Annual Meeting, Portland](#), Oregon**

**August 26-27, 2009, [2009 Soybean Rust Short Course](#), Quincy, FL**

**December 6-10, 2009, [NPDN National Meeting, Miami](#), FL**

**December 9-11, 2009, [2009 National Soybean Rust Symposium](#), New Orleans, LA**

**December 13-16, 2009, [2009 Entomological Society of America Annual Meeting](#), Indianapolis, IN**



[Mary McKellar](#), Editor  
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