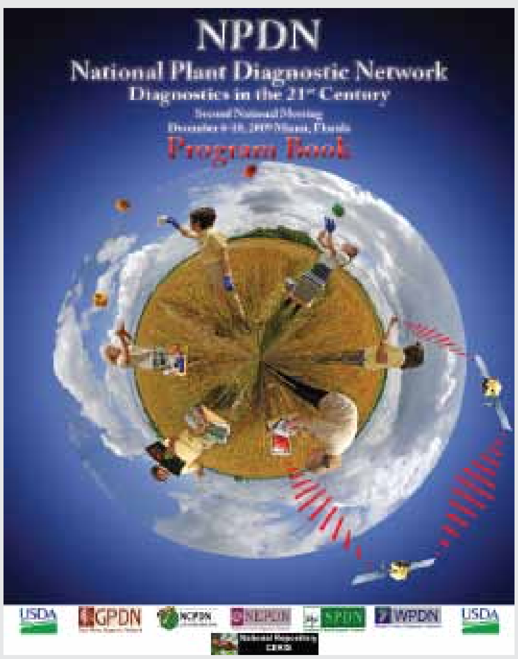
# NPDN News

NPDN National Plant Diagnostic Network
Volume 4 Issue 9, November 2009

## National Updates

### Second National Meeting of the NPDN December 6-10, 2009

In just a few days, we will gather in Miami, Florida for the 2nd NPDN National Meeting. Although the time seems to have passed quickly, we have accomplished a lot since we last met for a national meeting in January of 2007 and hopefully our development as a network will be evident during our meeting program and networking events. Many of you have chosen to get to Miami a little early and participate in the pre-meeting tour to Fairchild's Tropical Botanic Garden and the Miccosukee Indian Village. A welcome reception Sunday evening will get the meeting started. The program at this national meeting is packed full of interesting and thought inspiring presentations. First thing Monday morning, we jump right into subjects such as plant biosecurity, international trade, invasive pests, inspections at ports, and scientific questions about taxonomy challenges and the forensics of plant pathology. In the afternoon we continue with new and emerging threats, pests of regulatory significance, domestic communication, surveying, databases, and some specific pests and pathogens. We conclude our first day by gathering together for an enjoyable banquet on Monday evening. On Tuesday, you can look forward to a morning symposium on genomic tools and resources and an afternoon symposium on novel techniques for detection and surveillance. Also on Tuesday, we have some workshops




*Issue Highlights:*

- National Meeting Update
- Diagnostic Updates: Chrysanthemum White Rust
- The Proficiency Test Panel For 2010.
- Diagnostics Subcommittee Update
- Diagnostic Tip of the Month: A Novel Approach to Testing with Immunostrips
- Thousand Cankers of Black Walnut National Conference
- National Database Subcommittee Update
- National Repository: New Report Released
- Regional Updates
- Employment Opportunities
- Upcoming Events

planned. Tuesday morning, some of you may have signed up for the "Technology Tools and the NPDN" workshop and in the afternoon the "*Spodotera litura* and *S. littoralis* Identification" workshop. We have a busy evening planned for you Tuesday night, after a dinner on your own, with an International Session focusing on different networks and clinics and afterwards, sessions for plant pathologists to exchange information and entomologists to discuss widely prevalent lists. The regional meetings will be held on Wednesday and the post-meeting tour to the APHIS Plant Inspection Station, a couple of greenhouses, a tropical fruit winery, and the Tropical Research Education Center will wrap things up on Thursday. See you soon!

# Diagnostic Updates

## Chrysanthemum White Rust (CWR)

Chrysanthemum White Rust has been a significant problem in many states in recent years. Read here for a recent event in Virginia.

Chrysanthemum White Rust

### First find of Chrysanthemum White Rust in Virginia.

Chrysanthemum White Rust (CWR), caused by the fungus *Puccinia horiana*, was found at a Fairfax County retail nursery on September 24, 2009 by a Virginia Department of Agriculture and Consumer Services (VDACS) nursery inspector. Of approximately 171 chrysanthemums in the infected display block, 57 plants showed visible symptoms. All chrysanthemums that displayed symptoms at the nursery were shipped in from out of state. VDACS and APHIS-PPQ laboratories confirmed the samples were positive for CWR on September 28 and the retail nursery began eradication protocols the same day under direction of VDACS. All plants associated with the symptomatic plant block were destroyed. This is the first report of this plant disease in the Commonwealth of Virginia.

This disease originated in eastern Asia and has since spread throughout Europe, Africa, Australia, Central America and South America. There have been sporadic outbreaks in the past few decades in the United States and Canada, but so far the disease has been eradicated.

## The proficiency test (PT) panel for the 2010 *P. ramorum* National Plant Protection Laboratory Accreditation Program (NPPLAP) will be distributed from second week of November 2009 through February 2010.

The year 2009 marked the completion of five full years of NPPLAP inspections, training and proficiency testing in support of PPQ *P. ramorum* diagnostics. Like the 2008 and 2009 panel, the 2010 *P. ramorum* PT panel is a single format containing two sample types: 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 samples, please make sure that you have these permits up to date. Information and applications for permits can be viewed at the following

USDAAPHIS-PPQ website: [http://www.aphis.usda.gov/plant_health/permits/organism/index.shtml](http://www.aphis.usda.gov/plant_health/permits/organism/index.shtml).
As with the previous year's panel, the lyophilized tissue samples are designed to measure proficiency for two critical stages in the *P. ramorum* diagnostic process: the DNA extraction and the PCR analysis. *P. ramorum* positive and healthy DNA controls for PCR will be provided with each panel.

To ensure the PT panels are of sufficient quality prior to shipment, the samples are 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. Lyophilized tissue integrity (stability and uniformity) is ensured by extraction of DNA from randomly selected lyophilized tissue samples at regular time intervals and tested by real-time and conventional PCR.

As in 2009, the dual real-time PCR assay format will be an option for participating labs for 2010. Feedback from participating laboratories indicates that using the dual real-time PCR assays increases laboratory capacity and decreases the potential of cross-reaction. Please note: using the dual USDA-validated real-time PCR assays can render a valid diagnostic determination for most *P. ramorum* samples only when used concurrently. The dual real-time PCR assay format consists of the ITS-based real-time PCR (the assay used since 2006 in the program for diagnosis of *P. ramorum*) combined with a real time PCR targeting the Elicitin loci in *P. ramorum*. Both assays are in multiplex format using different internal control targets allowing a complete diagnostic determination to be made.

CPHST has documented that performance of the dual real-time PCR assays is not as sensitive for diagnosis of *P. ramorum* as the previously developed nested PCR assay, therefore inconclusive determinations still need to be resolved using conventional PCR, both nested and multiplex. Resolution of inconclusive samples can be accomplished by either conventional PCR or by forwarding DNA samples of inconclusive determinations to the USDA confirmatory lab.

For the 2010 *P. ramorum* proficiency program, diagnosticians seeking PT certification using conventional PCR alone (option C) or by using the ITS real-time and the conventional PCR (option R) will be required to perform the conventional PCR on all panel samples. Diagnosticians seeking PT certification using the dual real-time PCR format (option D) are only required to perform the ITS and Elicitin real-time PCR assays according to the work instructions. In contrast to the PT09 program, option D will no longer require the resolution of inconclusive samples by conventional PCR testing. Diagnosticians that select option D must identify the inconclusive samples generated during testing and will be required to indicate, by comment only, a further course of action for these samples.

Although opting for the dual real-time PCR format will no longer require proficiency in conventional PCR, it is highly recommended that laboratories that process large numbers of samples, labs that receive samples from other US states, and/or labs that make confirmatory diagnostic determinations demonstrate and maintain proficiency in the all available diagnostic options to maintain national capacity for sample determinations. Laboratories desiring PT certification in the dual real-time and conventional PCR assays should choose the option B that requires the performance of all four assays (conventional nested & multiplex and real-time ITS & Elicitin) on all PT panel samples. Certification under option B may be useful to many laboratories since demonstrated proficiency in the conventional PCR allows for the resolution of inconclusive determinations on-site and, in case of real-time PCR equipment failure or repair, serves as a back-up system during the testing season.

The option you select will dictate the method(s) you intend to use and therefore the primers/probes that you will need. We suggest that once you have made your choice, order primers/probes/reagents 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. Even though the primer and probes for these diagnostics are the same as those used for the 2009 program, 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 2010 PT panel are listed in tables (see link for tables). In addition, participating laboratories should not use DNA extraction kits that have expired since this may affect the quality of the DNA you extract and further the quality and outcome of your PCR analysis.

When you receive your PT panels, 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 a test evaluation and feedback within one month. Each individual participant should choose a preferred month to receive a panel(s) using the form (see link for form) and promptly return to the email address listed. We will make every effort 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 2010.

## Diagnostics Subcommittee Update

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

Since the last newsletter, the Diagnostics Subcommittee held two conference calls on October 22, 2009 and November 12, 2009. During these meetings, a number of issues were addressed. Please refer to the website, for complete minutes of this meeting www.npdn.org (login and password are required).

- Basic technique workshop and website design update
- Web-based platform update and possible adobe connect session
- IT/Diagnosticians meeting in review
- NPDN national meeting subcommittee poster
- Diagnostician training needs survey review
- NPPLAP *Phytophthora ramorum* proficiency testing.

The next conference call will be held on Thursday, December 17, 2009.

## A Novel Approach to Testing with Immunostrips

Gail Ruhl, Purdue University, Plant and Pest Diagnostic Laboooratory

# Diagnostic Tip of the Month

To simplify immunostrip testing in our lab we use racks, small plastic, disposable cuvettes (small test tubes work also) and disposable pipettes, as shown in the accompanying images. This eliminates the need to prop open the grinding bag to prevent wicking of liquid on the sides of the immunostrips and allows easy access to sample extract for multiple virus testing in one cuvette.

Small test tubes


Disposable pipettes

# Education and Training

## Thousand Cankers of Black Walnut National Conference

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

A very informative conference on thousand cankers of black walnut was held in St. Louis, Missouri on November 3-4, 2009. For two days, over 120 people from across the country, including Department of Agriculture personnel, extension educators, black walnut wood producers, black walnut nut producers, and others, gathered to discuss the thousand cankers disease and to discuss current and future research ideas, surveying events, outreach efforts and regulatory issues. The meeting agenda and (in time) proceeds can be found at the Missouri Department of Agriculture website, http://mda.mo.gov/plants/pests/thousandcankers.php. Ned Tisserat and Whitney Cranshaw of Colorado State University put together a pest alert that gives a great overview of the disease and beetle vector. Read more for their introduction to this pathogen and pest.


Large trunk cankers of black walnut associated with *Fusarium solani*

## Walnut Twig Beetle and Thousand Cankers Disease of Black Walnut

Within the past decade an unusual decline of black walnut (*Juglans nigra*) has been observed in several western states. Initial symptoms involve a yellowing and thinning of the upper crown, which progresses to include death of progressively larger branches. During the final stages large areas of foliage may rapidly wilt. Trees often are killed within three

Distribution of the walnut twig beetle. Green states and CA county of Los Angeles with records prior to 1992. States in orange have repported since 1998.

years after initial symptoms are noted. Tree mortality is the result of attack by the walnut twig beetle (*Pityophthorus juglandis*) and subsequent canker development around beetle galleries caused by a fungal associate (*Geosmithia* sp.) of the beetle. A second fungus (*Fusarium solani*) is also associated with canker formation on the trunk and scaffold branches. The proposed name for this insect-disease complex is thousand cankers.

For more of the pest alert, go to: http://www.ext.colostate.edu/pubs/insect/0812_alert.pdf

# Training Update

## NPDN-USDA AHPIS Potato Wart, *Synchytrium endobioticum*,

Karen L. Snover-Clift, Cornell University
Laurene Levy, USDA-APHIS-PPQ-CHPST-NPGBL
Kurt Zeller, USDA-APHIS-PPQ-CHPST-NPGBL

The NPDN Diagnostics Subcommittee and members of USDA-APHIS-PPQ-CHPST-National Plant Germplasm and Biotechnology Laboratory (NPGBL) collaborated to provide diagnostician hands-on, real-time PCR laboratory training for *Synchytrium endobioticum*, the causal agent of Potato Wart. Three training sessions took place during September and October of 2009. The NPDN offered this training to all its 52 State and Territory members and Plant Industry diagnosticians.

Kurt Zeller of the CPHST-NPGBL conducted the training which included background, and molecular lectures on *S. endobiotium*. Kurt was assited in individual sessions joined by NPGBL support scientists Liz Twieg and Gang Wei. John McKemy of USDA-APHIS-PPQ-NIS provided a lecture on morphology and taxonomy of *S. endobioticum.* Additionally, the participants were given the opportunity to conduct two types of hands-on, real-time PCR testing protocols targeting the ITS and 18S regions for the identification of *S. endobioticum*.

In all, twelve NPDN diagnosticians, State Department of Agriculture personnel and industry personnel from numerous states across the nation attended the three training sessions offered this Fall. The NPGBL is located in the PPQ facility in Beltsville, MD that most of us refer to as

October 6-8 training participants: Kurt Zellar (CPHST), Karen Snover-Clift, (Cornell Univ.), Karen Rane (Univ. of Maryland), Liz Twieg (CPHST), Bruce Watt (Univ. of Maine), Heather Faubert (Univ. of Rhode Island), and Joan Allen (Univ. of Connectict)

Not Pictured: September 15-17, 2009 training participants: Megan Kennelly, Kansas State University, Albert Patton, (Texas A&M University), Ram Sampangi (University of Idaho), Liz Vavricka (University of Idaho) and Nancy Taylor (Ohio State University).

October 20-22, 2009 training participants: Melodie Putnam (Oregon State University), and Elizabeth Schrum (Kansas State University).

"Building 580". Recent renovation to the NPGBL lab area has resulted in the NPGBL operating larger training classes (8-10 diagnosticians per session versus 6). PPQ is designing an addition to this facility that will increase the space for PPQ programs but has also planned for a dedicated set of training labs and a classroom for the lecture portion of training. If funding for the construction of this facility is approved it will result in increased NPGBL training sessions per year and larger class sizes when needed. As the NPGBL develops and validates new detection methods they will use these training sessions as a mechanism to transfer methods to diagnosticians in the NPDN, State Departments of Agriculture, and PPQ programs.

Upcoming training sessions:
Laurene Levy and her NPGBL colleagues are planning to continue this very valuable collaboration between USDA-APHIS and NPDN and offer new workshops in the Spring of 2010. We are in the planning stages of developing the workshops for next year and will send out detailed information as soon as it is available. If you are interested in participating in any of these workshops, please contact Karen Snover-Clift at kls13@cornell.edu.

## Nucleic Acid-Based Pathogen Detection Workshop

A hands-on workshop for applied plant pathologists on nucleic acid-based pathogen detection will be held at the University of Kentucky in Lexington on 19-22 Jan, 2010. We have space available for one additional participant. Participants will design, execute, and interpret at least three real-time PCR experiments (both SYBR Green and Taqman assays, including an assay for pathogen quantitation); extract DNA; run a dot-blot experiment for amplicon verification; run analytical and preparative agarose gels; and interpret DNA sequence data from one of their PCR experiments. Presentations include theory of real-time PCR, experimental controls, PCR inhibition, use of PCR kits, verifying amplicon identity, licensing, sample quantitation, arrays, minimizing contamination, and troubleshooting. Primer design is not included in the workshop. For more information, contact Paul Vincelli (pvincell@uky.edu).

# National Database

## New Report Release From the NPDN National Repository

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

Collaborations with Carla Thomas from UC Davis have resulted in the development of a new first submission report which has been made available to regional staff and national program leaders. The report contains the previous day's summaries of pests, weeds, or diseases submitted to the NPDN Repository for the first time in a state. This tool sends out the region's first submission reports on a daily basis to regional NPDN staff with authorized access to better monitor potential anomalies. For more information please contact Mike Hill at (765)494-9854 or mhill@ceris.purdue.edu.


Purdue University, West Lafayette, IN

## National Database Subcommittee Update

Karen L. Snover-Clift
Committee Chair
Cornell University

Since the last newsletter, the National Database Subcommittee held a conference call on November 11, 2009. The subcommittee 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 website, http://www.npdn.org/national_database, for complete minutes of this meeting. (login and password is required).

- Discussion of change submission
- Discussion of adding two new diagnosticians to the group
- NPDN National Database subcommitee national meeting poster
- Additional Phase 2 fields
- Discussion of fungi pest beginning with scientific names G, H, and I

The next meeting will be held on December 16, 2009.

# Regional Updates

WPDN
Western Plant Diagnostic Network

## Western News

Recent WPDN Pest Alerts:

### European Grapevine Moth, *Lobesia botrana*: A New Pest in California

*Lobesia botrana*, European grapevine moth was first reported in the United States from Napa County vineyards in October 2009. Native to Southern Italy, it was first described from Austria and is now found throughout Europe, North and West Africa, the Middle East, and eastern Russia. It was more recently introduced into Japan, and in 2008, it was first reported in Chile. It belongs to the family Tortricidae, sub-family Olethreutinae. Earlier species names included *Polychrosis botrana* and *Eudemis botrana*. In Europe, some of the common names are eudemis (France); tignolleta della vite (Italy); bekreuzter

traubenwickle (Germany); polilla del racimo (Spain); and European grape berry moth and European vine moth (English-language literature). Read more at http://www.ipm.ucdavis.edu/EXOTIC/eurograpevinemoth.html

### Spotted Wing Drosophila:

Spotted wing drosophila has recently been found in many California counties infesting ripening cherry, raspberry, blackberry, blueberry, and strawberry crops; it has also been observed attacking other soft-flesh fruit such as boysenberry, varieties of Japanese plums, plumcots, and nectarines. Adults and maggots closely resemble the common vinegar fly, *Drosophila melanogaster*, and other Drosophila species that primarily attack rotting or fermenting fruit. The spotted wing drosophila, however, readily attacks undamaged fruit.

Adult female spotted wing drosophila, *Drosophila suzukii* Photo by Martin Hauser

Adults are small (2-3 mm) flies with red eyes and a pale brown thorax and abdomen with black stripes on the abdomen. The most distinguishable trait of the adult is that the males have a black spot towards the tip of each wing. Larvae are tiny (up to 3.5 mm), white cylindrical maggots that are found feeding in fruit. One to many larvae may be found feeding within a single fruit. After maturing, the larvae partially or completely exit the fruit to pupate.

The spotted wing drosophila can be distinguished from the western cherry fruit fly, *Rhagoletis indifferens*, by comparing anatomical features of the maggots and wing patterns of adult flies. Western cherry fruit fly adults are much larger (5 mm) than the spotted wing drosophila adults and have a dark banding pattern on their wings. The western cherry fruit fly, which is a quarantine pest, occurs in Washington and other states but has not established in California. If you suspect you have a western cherry fruit fly, take specimens to your local agricultural commissioners' office. Read more here: http://www.ipm.ucdavis.edu/EXOTIC/drosophila.html

# Employment Opportunities

## Missouri Department of Conservation

The Missouri Department of Conservation is seeking candidates for the Forest Pathologist (Resource Scientist) position located in Columbia, Missouri, USA. The Pathologist partners with the Forest Entomologist to monitor forest health and provide forest health information to resource managers, landowners and the general public in Missouri. The Pathologist provides leadership in Department responses to emerging forest disease issues and conducts forest pathology research relating to forest health monitoring and resource management. The announcement and information for applying online are available at this site: http://mdc.mo.gov/about/jobs/

## Extension Support Specialist

**Department of Plant Pathology & Plant-Microbe Biology within the College of Agricultural and Life Sciences**

The Department of Plant Pathology and Plant-Microbe Biology seeks a highly motivated to serve the educational and communication needs of the Northeast Plant Diagnostic Network (NEPDN), which is a part of the National Plant Diagnostic Network (NPDN) funded by the USDA National Institute of Food and Agriculture. The appointee will be a member of a team based in the Department of Plant Pathology and Plant-Microbe Biology at Cornell University, Ithaca, NY. For more information e-mail Karen Snover-Clift at kls13@cornell.edu

# Upcoming Events

## National Events

**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

**January 19-22, 2010**
Hands on workshop for applied plant pathologists on Nucleic acid-based pathogen detection.
Lexington, KY

**May 18-20, 2010**
NPDN Diagnostician Basic Technique Workshop
The Pennsylvania State University
State College, PA

## Regional Events

**April 6-7, 2010**
NCPDN Regional Meeting
St. Paul, MN

Editor
NEPDN
Cornell University