NPDN National Plant Diagnostic Network

NPDN News

Volume 3 Issue 6, June 2008

National Updates

Provide Your Input on NPDN 2009 National Meeting Associated Programs

The NPDN National Meeting Planning Team, Associated Programs Subcommittee members are planning pre- and post-meeting tours for the December 2009 NPDN National Meeting.

One of several options for associated program tours: Kerry's Greenhouse, Bromeliads. Photo Karen Snover-Clift

We are trying to determine how many people might be interested in pre- and post-meeting tours so we can plan accordingly.

We have created a very short (4 questions) survey. Please take five minutes to help us make plans for our national meeting by completing the survey. The survey was mailed to the NPDN Newsletter mailing list on June 23, 2008. If you did not receive the email, you can get to the survey through this link:
http://www.surveymonkey.com/s.aspx?sm=xhht6q3_2feIFWVDbyeIjjcQ_3d_3d

Please complete the survey by the deadline of **July 15, 2008**. Thank you for your help.

Issue Highlights:

National Updates

Oklahoma State University Conducts Plant Health Exercise

The National Institute for Microbial Forensics and Food and Agricultural Biosecurity (NIMFFAB) at Oklahoma State University recently conducted the first of many planned plant health exercises.

Participants in the exercise included representatives from the Oklahoma Cooperative Extension Service, the Oklahoma Agricultural Experiment Station, the OSU College of Osteopathic Medicine, the Oklahoma Department of Agriculture, Food and Forestry, the National Plant Diagnostic Network, the Animal Plant Health Inspection Service of the U.S. Department of Agriculture, the Department of Homeland Security, the FBI and the Department of Defense.

The four day event consisted of classroom work as well as hands-on learning and a facilitated discussion. The first day of the exercise participants were taught about plant pests and diseases and criminal investigative methodology, as well as the various key response agencies. On the second day of the exercise, participants spent half of the day out in the field. Through practicing a scenario of an introduced pathogen in a crop, participants learned how to examine a site for forensic evidence, to collect and preserve plant samples while maintaining chain-of-custody, to interview farmers and crop consultants as well as the capabilities, availabilities and limitations of mobile lab facilities. The remainder of the day included a tour of the NPDN lab, and a hotwash, reviewing what had been learned in the field including chain of communication in the event of the discovery of an introduced pathogen. The third and fourth days were spent in a facilitated discussion of the roles and responsibilities of agencies and organizations during an intentional criminal attack on plant health. Activation and notification as well as mutual aid and resource availability was also discussed.

Carla Thomas, NPDN National Exercise Coordinator facilitated the two day discussion. The situation manual was developed by APHIS-PPQ Professional Development Center. The event was sponsored by the Oklahoma Department of Homeland Security.

Diagnostic Updates

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 June 12, 2008. 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:

- Appointment of new secretary- Sara May, Penn State University.
- Diagnostics matrix submission for NPDN strategic plan.

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- Future Beltsville-NPDN diagnostician training.
- Lab accreditation progress.
- National meeting update.
- Lepidoptera workshop in the planning stage.

The next conference call will be held on July 17, 2008.

Diagnostic Tip of the Month: Obtaining Pure Bacterial Colonies and an Alternative to the Gram Stain

Melodie Putnam
Oregon State University

Starting with an axenic bacterial culture is a requirement for any sort of valid identification work. Individual colonies can look pure but actually be a mixture of different isolates of entirely different genera that grow at different rates – the contamination may not show up for weeks, but by then the plates have usually been thrown out.

In our lab, we work with a lot of slow growing bacteria and it is essential that we get good separation of colonies when we streak plates. To reduce the amount of contaminating and competing bacteria, we have adopted a technique that quickly allows us to obtain well separated pure bacterial colonies.

The method is simple: we add a water dilution step each time we re-streak our isolates. Specifically, from a colony we desire to purify, we will touch our flamed and cooled inoculation loop to the colony to pick up cells, and then dislodge the bacteria into a tube of 3 ml sterile deionized water. We then vortex the tube, and streak out as usual doing a standard dilution streak. We do this a total of three times, and usually by the last streak we have well separated pure colonies.

In the rare instance where the colonies still appear mixed, we will continue with the water/dilution streaks until a single colony type is present. We can verify purity when we do the Gram stain, as mixed cultures are often indicated by different morphologies.

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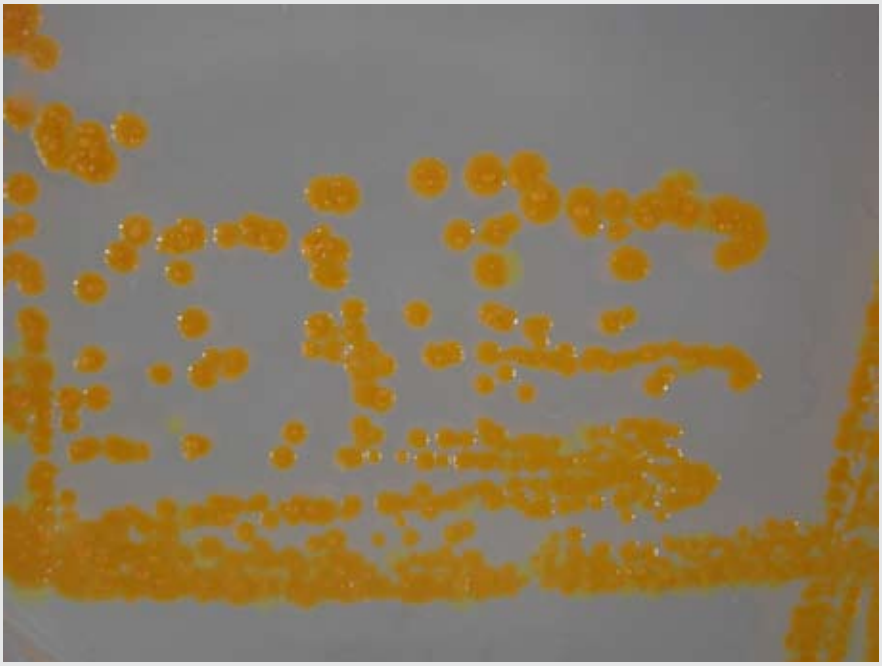

Close-up of a mixed bacterial colony. Photo Melodie Putnam

Diagnostic Tip of the Month

Diagnostic Tip of the Month

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For those of you who don't like to do Gram stains, there is another method that can be used to check the Gram reaction, which uses KOH instead of dyes. This test (as does the Gram stain) relies on the differences in the cell wall chemistry between the two types of bacteria. Gram negative cell walls are lysed by 3% KOH and release their contents, including the DNA. The DNA/KOH mixture is viscous and will form a thin, mucoid strand when a needle or other slender object touches and then is pulled away from the disrupted cell mass (photo). Gram positive cells are not affected by the dilute alkali solution and will not lyse, hence no viscous string will form.

This test requires a visible number of cells (a good clump), a sterile toothpick or inoculating loop, a clean slide, and 3% KOH. Cultures should be less than five days old (Halebian, et al., 1981).

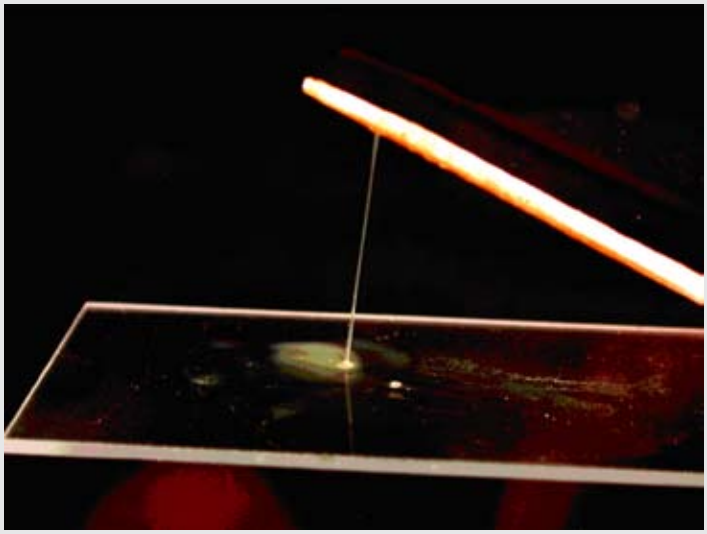
The procedure:

1. Place two drops of 3% KOH on a clean glass slide. Slides with wells in them work well for this.
2. Pick up a load of bacteria from a culture plate using a sterile toothpick or flamed and cooled inoculating loop.
3. Mix the bacteria in the KOH by stirring, and lift the toothpick or loop 1-2 cm from the slide after about 15 seconds.
4. Production of a viscous string within 30 seconds is indicative of Gram negative bacteria. Gram positive bacteria will not form a viscous slime.

NOTE: This assay is not reliable for characterization of certain anaerobic Gram negative bacteria (e.g. species of *Bacteroides*, *Fusobacterium*, *Leptotrichia*, and *Veillonella*). Other methods should be used for typing anaerobic bacteria.

Reference cited

Halebian, S., Harris, B., Finegold, S.M., and R.D. Rolfe. 1981. Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. Journal of Clinical Microbiology 13:444-448.



A viscous string of slime from Gram negative bacterial cells treated with a few drops of 3% KOH. The cells were added to the KOH on a slide, and mixed with a sterile toothpick. Gram positive cells will not form this type of ropy slime. Photo Melodie Putnam

Education and Training

Updates on First Detector Training in the WPDN

WPDN Holds a First Detector Educator Conference in Arizona

The WPDN held a First Detector Educator conference at the Maricopa Agricultural Center in Arizona on May 27, 2008. There were 35 participants from the University of Arizona, University of Arizona Cooperative Extension, the USDA-PPQ, and private industry. The three modules for First Detector training, tailored for Arizona agriculture and horticulture, were presented by Dick Hoenisch and discussed by the audience as to the content and adaptability of the Power Point modules for specific groups. Dick Hoenisch also showed the various NPDN websites to demonstrate the resources that are available on these and other websites. For the last segment of the training session, the new, on-line NPDN modules were demonstrated. The group went over the new modules: Diagnosing Plant Problems and Photography for Diagnosis. With this "Training the Trainer" workshop, Arizona's First Detector program is off to a good start.

Dick Hoenisch, WPDN Training Coordinator, giving presentation at recent first detector training in Reno, Nevada.

WPDN Conducts Long Distance First Detector Training in Nevada

Dick Hoenisch traveled to Reno, Nevada to conduct a First Detector training session on June 4, 2008, with Shouhua Wang, Plant Pathologist for the University of Nevada Cooperative Extension and First Detector training coordinator for Nevada. There were 25 participants present at the Cooperative Extension Center in Reno, and another 27 participants in 5 locations around the state.

The Center has an excellent tele-conferencing system organized by Leslie Allen, the Commercial Horticulture Program Coordinator. All the participants in the remote sites had received the First Detector training packets by FedEx, and could fully participate in the conference, including asking questions. It was quite a useful and impressive mode of conducting training. The will be a similar conference with remote locations in Las Vegas on June 19.

Participants at the Nevada first detector training.

National Database

National Database Subcommittee Update

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

The NPDN national database subcommittee met on June 11, 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 national database subcommittee web page of the NPDN web site for complete minutes of this meeting (login and password required).

Asian citrus psyllid, *Diaphornia citri*. Photo David Hall, USDA-ARS, www.ipmimages.org.

Topics of discussion included:

- Change submission requests.
- Fungal disease common names beginning with the letter C and H,I.
- The next batch of common names to review.

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

Regional Updates

SPDN
Southern Plant Diagnostic Network

Southern Region Detection of Asian citrus psyllid, *Diaphornia citri* Kuwayama and Citrus Greening, in Louisiana

On May 29, 2008, the USDA-ARS Systematic Entomology Laboratory in Beltsville, Maryland, confirmed the identification of a specimen of Asian citrus psyllid (ACP) from a residential property in Algiers, Orleans Parish, Louisiana.

The psyllid was first collected by the homeowner, who submitted a digital photograph to the Louisiana State University (LSU) Agcenter Extension Entomologist. After review of the photo LSU Agcenter notified the Louisiana Department of Agriculture and Forestry (LDAF) of the suspect psyllid. APHIS then visited the property and collected specimens.

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The psyllid was found on a lime tree that has been on the property for approximately 7 years, and planted in the ground for at least 3 years.

On June 12, 2008, the USDA Animal and Plant Health Inspection Service Plant (APHIS), Plant Protection and Quarantine (PPQ) Molecular Diagnostics Laboratory and the Center for Plant Health Science and Technology (CPHST) National Plant Germplasm and Biotechnology Laboratory in Beltsville, Maryland, confirmed the identification of citrus greening (CG, also known as Huanglongbing or HLB) in a leaf sample from a residential property in Algiers, Orleans Parish, Louisiana. HLB is caused by the bacterial pathogen '*Candidatus* Liberibacter asiaticus'. This is the first confirmation of CG in Louisiana.

The samples in which CG was confirmed were from a lime tree on which Asian citrus psyllid (ACP, *Diaphornia citri* Kuwayama) had previously been found.

For more information on these current detections, please visit on the web:

NAPPO Phytosanitary Alert System: Detection of Asian citrus psyllid, *Diaphornia citri* Kuwayama, in Louisiana

NAPPO Phytosanitary Alert System: Confirmation of Huanglongbing or Citrus Greening ('*Candidatus* Liberibacter asiaticus') in Louisiana – United States

Regional Updates

WPDN
Western Plant Diagnostic Network

Western Region
Japanese Dodder, a New Phanerogamic Pathogen in California

Timothy Tidwell
Plant Pathologist/Program Supervisor
CDFA Plant Pest Diagnostic Center

Japanese dodder. Photo Barry Rice, www.ipmimages.org.

In the United States, there are about 50 species of Cuscuta, the parasitic plants known as "dodder," some of which are native and some are invasives. Dodders are true parasites, usually having yellow to orange thread-like stems lacking true leaves and chlorophyll, and parasitize host plants via haustoria. The dodders produce flowers and seeds and also vegetatively propagate via fragmentation of the stems. Most dodders are not particularly serious agricultural pests, although in a few cases some species can significantly reduce crop yields in hosts such as alfalfa if infestations become large.

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Regional Updates

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Japanese dodder, *Cuscuta japonica* Choisy, on the other hand, is an exotic, invasive dodder recently detected in California. It is a robust, fast-growing native of Asia with a wide host range that has been detected throughout California over the last four years.

How significant is it?
Japanese dodder is considered a "noxious weed" on both Federal and California noxious weed lists. But it is far more sinister than a mere weed. This phanerogamic parasite, the stems of which are capable of growing six inches per day once established on a host, has the ability to quickly parasitize, weaken, smother and kill its hosts, as well as vector plant pathogens such as viruses, bacteria, and several phytoplasmas.

Japanese dodder has a wide host range that includes woody and herbaceous plants. These include both native plants and many commercially important taxa such as trees in the Roseaceae (apples & various *Prunus* species), citrus, grapes, as well as numerous ornamental hosts--- important in a state where nursery crops represent a significant portion of agricultural production.

Where is it and where did it come from?
Japanese dodder was initially detected on a citrus tree in Northern California in 2004, and is now known from more than 200 California sites ranging from North Central to Southern California. It is native to the temperate coastal areas of Asia including regions of China, Korea, Japan, and Russia. In the U.S., Japanese dodder has already become a nuisance pest in Florida, South Carolina, and Texas, particularly in the city of Houston.

How does it spread?
Japanese dodder is relatively hardy in temperate to warm climates. Although it is usually considered an annual, in California Japanese dodder has the potential to overwinter in and on its host and resume growth in spring, thus behaving more like a perennial.

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A 25-foot tall tree in a residential neighborhood of Sacramento, CA extensively parasitized by Japanese dodder. Photo by Terra Irving, CDFA Plant Pest Diagnostic Center.

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In regions of California that have very mild, frost-free winters, vegetative growth often continues year-round resulting in enormous amounts of dodder biomass.

Although Japanese dodder does flower in California, after four years it has yet to actually produce viable seed at any infestation site. This may be due, at least in part, to the fact that flowering in California occurs in late autumn to early winter, leaving essentially no time for seed to mature even if it were to set. In addition, the plant appears to be self sterile, based on self-pollination studies by Dr. Hrusa in the greenhouse. However, when plants from two different regions were experimentally brought together and cross-pollinated in the greenhouse, Dr. Hrusa was able to get a few seed to form, suggesting that there may be more than one clone (individual) present among the infestations in California.

For a number of reasons, however, seed production may not be particularly important in the survival and spread of this pathogen in California. Japanese dodder grows rapidly, producing huge amounts of biomass capable of smothering large trees (Figure 1) with large amounts of dodder stems, all potentially capable of fragmenting and starting new infections.

In addition, the parasite over winters well---particularly in regions that lack freezing weather where the plants may grow indefinitely. Wildlife, most notably birds, can move vegetative pieces to the tops of trees where infestations can become established out of sight among the canopy of leaves, making early detection difficult. And finally, there is good evidence that human dispersal, propagation, and "cropping" is largely responsible for the introduction, as well as the extensive and quick spread of the parasite in California. It is suspected that Japanese dodder was deliberately introduced, not only in California, but also in the Southeast USA as well, for the purpose of herbal medicine.

Regional Updates

Management Strategies in California

Detection of Japanese dodder sites has consisted primarily of surveying residential neighborhoods, since that is the only environment in which to date, the parasite has been found in California. More recently surveys have focused more on neighborhoods known to have high populations of immigrants who might be likely to use the dodder for its medicinal properties. Although eradication efforts at the various infection sites have been largely successful, new infestation sites continue to be detected at a discouraging rate. More efficient and more expedient methods of detection are needed. Aerial surveys have been proposed but are still in the planning stage.

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Upcoming Events

National Events

July 26-30, 2008, Centennial APS/SON Joint Meeting, Minneapolis, MN

August 10-14, 2008, National Plant Board 2008 Annual Meeting, Solomons, Maryland

August 18-20, 2008, Mite Taxonomic Workshop, Gainesville, FL

November 16-19, 2008, ESA Annual Meeting, Reno, NV

March 24-26, 2009, Sixth International IPM Symposium, Portland, OR

December 6-10, 2009, NPDN National Meeting, Miami, FL

NPDN
National Plant Diagnostic Network

CSREES
USDA

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