NPDN National Plant Diagnostic Network

NPDN News

Volume 3 Issue 5, May 2008

National Updates

First Detection of Soybean Rust on Coral Bean in U.S.

During the last weeks of April this year, soybean rust was detected on a new host in the U.S. Since this was a detection of soybean rust on a previously unreported host in the U.S., final confirmation of the disease was made by the USDA-APHIS-PPQ National Plant Germplasm and Biotechnology laboratory in Beltsville, MD. The sample was collected in a known positive patch of kudzu in Marion County, Florida. The new host, coral or Cherokee bean (*Erythrina herbacea*) is found widespread in Florida and other southern states.

Eastern coral bean. (Photo Chris Evans, River to River CWMA. www.ipmimages.org)

This legume is used in gardens and can be seen growing on roadsides. It can overwinter in frost free areas of Florida including the central part of the state and south of Tampa.

For more information about this detection and the status of soybean rust in the U.S., please visit on the web www.sbrusa.net.

Status of Citrus Greening in the U.S.

In September 2005, Citrus Greening ('*Candidatus* Liberibacter asiaticus') was detected in both homeowner yards and commercial citrus groves within the state of Florida. Subsequent surveys within the state determined that the disease was well established statewide.

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Issue Highlights:

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As a result, the USDA has issued Federal Domestic Quarantine Orders that restrict the interstate movement of host plants and plant parts including seeds but excluding fruit from leaving the state. To date, the disease has not spread outside of Florida.

For more information on this pest and its current status, please visit on the web:

NAPPO Phytosanitary Alert System: Status of Huanglongbing or Citrus Greening (‘*Candidatus* Liberibacter asiaticus’) in the United States

Cogongrass Workshop, July 22, 2008, Nashville, TN

Cogongrass is widely considered to be the worst invasive plant threat in the Southeastern U.S. It invades forests, pastureland and rights of way. It is highly flammable, a highway hazard and destroys wildlife habitat and recreational value of land. Eradication can be costly; up to $200 per acre. Cogongrass was confirmed by the USDA in Tennessee on April 15, 2008.

In response to the confirmed presence of cogongrass in Tennessee, the Tennessee Exotic Pest Plant Council will be coordinating a workshop to be held on July 22, 2008 from 10am to 2:30pm in Nashville, TN. If you are interested in attending this workshop please contact Anni Self at Anni.Self@state.tn.us or 615-837-5313.

Infestation of cogongrass in pine reforestation. (Photo Charles T. Bryson, USDA-ARS, www.ipmimages.org)

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 May 8, 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:

- Future Beltsville-NPDN diagnostician training.
- Permit instructions update.
- Suggestions for future diagnostician-IT needs.
- Lab accreditation progress.
- National meeting update.
- NPDN diagnostics matrix.

The next conference call will be held on Thursday, June 12, 2008.

Diagnostic Tip of the Month

Double-Stranded RNA Analysis as a Tool for Diagnosing Plant Viruses

J.R. Fisher
Plant Pathologist
Ohio Dept. of Agriculture

Diagnosing plant viruses can be a challenging endeavor. The number of viruses infecting plants is formidable and previously uncharacterized viruses are being described regularly, thereby adding to the challenge.

How, then, does a diagnostician confronted with virus-like symptoms approach identifying the causal agent? Viral symptoms are often striking, but rarely are they specific to a particular virus. Likewise, a particular virus often produces very different symptoms on different hosts.

Fortunately tools such as ELISA (enzyme-linked immunosorbent assay) and PCR (polymerase chain reaction) exist which are very useful for virus identification, but they require that you at least have some idea of which virus(es) you are dealing with. ELISA and PCR as the first line of virus identification are a shot in the dark at best due to the specificity of the assays. They also may allow a mixed infection to go undetected due to their specificity. So where does one turn?

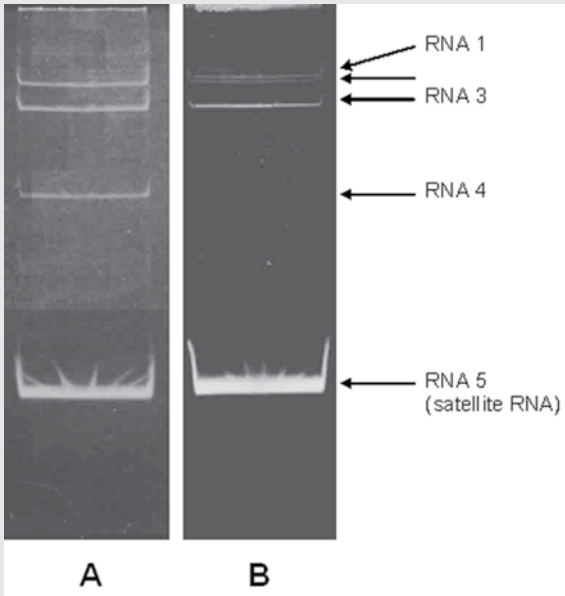

Fig.1. dsRNA profiles from: A) cucumber mosaic virus (CMV) infected *Lobelia* spp. and B) CMV infected *Ajuga reptans*. CMV genomic dsRNAs 1-3 and subgenomic dsRNA 4 indicated by arrows (Note: RNA 4 appears absent in gel B). A satellite RNA (dsRNA 5) is also indicated. The Lobelia sample is also likely infected with at least one other virus as suggested by additional dsRNAs in the gel. Gel is 5% polyacrylamide stained with ethidium bromide. Electrophoresis was performed at 125 volts for 90 minutes.

Double-stranded ribonucleic acid (dsRNA) analysis is a non-specific alternative to more precise assays like ELISA and PCR. The majority of viruses infecting plants (>95%) are composed of single-stranded messenger sense (+) RNA genomes. When these ssRNA+ viruses replicate in the host cell they go through an intermediate double-stranded stage where a negative sense (-) RNA strand is copied from the sense strand. This antisense strand serves as the template for synthesis of more messenger sense RNA. It is the intermediate double-stranded RNA molecule that is useful as a diagnostic tool.

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Diagnostic Tip of the Month

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Interpretation of the results is the key. In the case of a virus with a multi-partite genome the dsRNA banding profile may provide a clue as to the viral genus. This is true of the cucumoviruses (Fig. 1) and alfamoviruses. Likewise, the relative molecular weight of a monopartite genome may point you toward the viral genus or group.

It is important to be aware of what you might be seeing in a dsRNA gel. A monopartite virus may produce one or more sub-genomic dsRNAs in addition to the genomic dsRNA. The same is true for a multipartite virus. Even if the dsRNA banding profile isn't familiar to you, at least it is indicative of a viral infection and you may be able to narrow the field by searching the literature for clues. Or you may not see anything. In that case the plant may still be infected with a virus but it could be a DNA virus or a ssRNA- virus. It is important to note that although most uninfected plants do not produce dsRNAs there are some, like the cucurbits, that produce indigenous dsRNA molecules. It is therefore imperative to include 'healthy tissue' controls so you don't mistake indigenous dsRNAs for viral dsRNAs. As with many techniques, the more experienced you become with dsRNA profiles the more comfortable you will become interpreting the results.

dsRNA analysis has advantages and disadvantages. Advantages include it is relatively inexpensive, making use of fairly common lab reagents and equipment. It is also relatively quick, a typical sample being prepared from start to finish in two days (there are tricks to shorten the time frame). The dsRNA molecule is very stable, so precautions that one would take when working with messenger RNA (ie. RNAse-free environment) need not be so stringent. The purified dsRNA molecule can also be used as template for cDNA synthesis and subsequent PCR.

A disadvantage is that this procedure would generally not be used to process a large number of samples at a time (16 is the most I have attempted at once). Also, since dsRNA is related to replication of the virus the titre of the dsRNA molecule(s) may increase or decrease in the plant at certain times of the year, thereby influencing detection. Summer is generally an unfavorable time of year for plant growth and virus replication due to the heat. It's also important to note that this technique doesn't work for all ssRNA+ virus groups, notably the ilarviruses. It also doesn't work for the ambisense tospoviruses (Tomato Spotted Wilt and Impatiens Necrotic Spot viruses), but does work well for the cucumoviruses, alfamoviruses, tobamoviruses, potyviruses, potexviruses, and closteroviruses.

dsRNA analysis has become an unsung tool for diagnosing plant viruses, but one that is still useful because of its non-specific nature. Even given its limitations I believe it still has a place in the diagnostic and research laboratory. At the very least it provides a jumping-off point in the diagnostic process before proceeding to more specific techniques.

For protocol information contact:
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Education and Training

Maine First Detectors Help to Search for Summer Fruit Tortrix Moth

Maine first detectors will be volunteering their time this summer to help with a statewide survey for Summer Fruit Tortrix Moth (*Adoxophyes orana*).

This survey is a cooperative venture between the University of Maine Cooperative Extension, the NPDN and APHIS-PPQ.

The survey will consist of trapping moths at thirty locations across the state. This is the first time that APHIS-PPQ has enlisted the assistance of the public in a survey in Maine. Depending upon results of this survey as well as the interest of the participants, projects with first detectors in Maine may be expanded in the future.

Adult summer fruit tortrix moth. (Photo Jae-Cheon Sohn, www.ipmimages.org)

Volunteers Needed for Booth at 2008 NACAA Meeting

This year's NACAA meeting will be held July 13-17, 2008 in Greensboro, North Carolina. We will have a NPDN First Detector educational booth that will highlight the launch of the Online Crop Biosecurity Program and other NPDN resources available to educators.

The exhibit will be available during the following dates and times:
July 13, 2008: 1:00pm-6:30pm
July 14, 2008: 8:00am-6:00pm
July 15, 2008: 8:00am-6:00pm

If you are planning to attend the meeting and would be willing to volunteer a few hours of time at the booth, please contact Amanda Hodges, achodges@ufl.edu.

Regional Updates

NEPDN
Northeast Plant Diagnostic Network

Northeast Region

National Repository Training: Degree Day Maps

The NEPDN will host a training via Adobe Connect on the National Repository feature of degree day mapping on **June 15**. The training is available to diagnosticians and will cover how to view their lab's data overlaid with degree day data generated by Oregon State. Interested in Patricipating? Contact Mary McKellar (mem40@cornell.edu).

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

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

Education and Training Events

June 15, 2008, NEPDN National Repository Degree Day Mapping Training, via Adobe Connect, contact Mary McKellar for more information.

June 22-25, 2008, Natural History and Taxonomy of the Carabidae, Oak Lake Field Station, South Dakota, contact Jonathan Lundgren, jonathan.lundgren@ars.usda.gov for more information.

June 24-27, 2008, 2008 eXtension National Community of Practice Meeting, Louisville, KY.

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