NPDN National Plant Diagnostic Network

NPDN News

Volume 6 Issue 9, September 2011

NPDN Participates in Department of Homeland Security (DHS) Tabletop Exercise (TTX) on *Rathayibacter toxicus*

Marie L. Socha, Contract Support to DHS Science & Tech Directorate, SHRR Consulting, Inc.; Kitty Cardwell, USDA-NIFA; Pat Sheil, USDA-APHIS and Karen Snover-Clift, Cornell University

On September 14 and 15, 2011, the National Plant Diagnostic Network, a member of the DHS Integrated Consortium of Laboratory Networks (ICLN), participated in a two-day tabletop exercise which focused on intentional contamination of fields in Kansas, Colorado and Wyoming with the plant pathogen, *Rathayibacter toxicus*. The exercise was performed as part of the ICLN's regular practice of evaluating the ICLN Network Coordinating Groups (ICLN NCG) Standard Operating Procedure.

During the two day exercise, mock situational injects were provided to NPDN representatives and they were then evaluated on how they utilized the ICLN portal, as well as, how they alerted fellow ICLN NCG members using either a Preparedness Alert (if an incident has not been confirmed) or a Situation Report (if an incident has been analytically confirmed), and finally how they convened conference calls to define whether they could handle the analytical capacity which resulted from sampling efforts or if they needed to request assistance from a fellow ICLN network. Other activities included utilizing the ICLN portal to upload and merge minimum data element worksheets (a form of data reporting sheet which can be merged with other data sheets).

Planning activities associated with this exercise helped to define informational gaps in sampling and analytical methodologies. One of several gaps found includes the lack of an approved method for which to analyze *R. toxicus*. Since *R. toxicus* is endemic in Australia, we requested their recommendations for *R. toxicus* identification testing procedures and sample collection methods for field situations and harvested products.

The most significant benefit of this exercise was collaboration between fellow ICLN-members, including Environmental Protection Agency's (EPA) Environmental Response Laboratory Network and USDA's

Issue Highlights

USDA National Institute of Food and Agriculture

National Animal Health Laboratory Network (NAHLN). During these collaborative efforts, EPA was able to provide information about sampling resources which could be used by NPDN for mass sampling assistance while NAHLN was able to inform NPDN of their ability to provide analytical support for times when NPDN exceeded their normal laboratory capacity. This exercise illustrated how the consortium of laboratories can effectively collaborate during an animal and plant disease outbreak.

Daylily Leafminer, *Ophiomyia kwansonis* Sasakawa, Newly Identified in North America

Gaye L. Williams, Plant Protection & Weed Management, Maryland Department of Agriculture and Gary J. Steck, Florida Department of Agriculture and Consumer Services, Division of Plant Industry

Since at least 2008, daylily growers and collectors have been aware of an unknown leafminer damaging leaves of *Hemerocallis* spp. and varieties (see comments at www.daylilies.org/ahs_dictionary/leafminer.html). In late 2008, at a Maryland daylily garden, Williams noted leafmining damage but found no live larvae. Attempts to rear flies from this site in 2009 and 2010 were unsuccessful. In June 2011, adults reared from puparia collected at the site were tentatively identified as *Ophiomyia kwansonis* Sasakawa. Collected larvae, pupae, adults and reared specimens were sent to Dr. Owen Lonsdale, Canadian National Collections, who confirmed their identity as *O. kwansonis*, a species previously known only from Japan and Taiwan. An image, www.bugguide.net/node/view/84826, from 2006, shot in Maine on daylily, carries Dr. Lonsdale's 2008 comment to that effect.

Also in 2011, Steck collected and identified Florida specimens of *O. kwansonis*. For more details see: Steck, G.J. and G.L. Williams, 2011, Florida DACS, DPI-Pest Alert – "Daylily leafminer, *Ophiomyia kwansonis* Sasakawa, (Diptera: Agromyzidae), new to North America, including Florida" (in prep/review).

Daylily leafminer is most easily detected by the long meandering, serpentine mines caused by larval feeding in the leaf blades (Fig. 1). Mines are usually seen in upper leaf surfaces, but in higher populations or reinfestations, may also occur in lower surfaces. One to several larvae may be found in a single leaf. The pale yellow larvae (Fig. 2), up to 5mm, can be detected in plant tissue with a 10x hand lens, usually by spotting the black mouthparts as they rake back and forth in the mine, feeding on plant tissue

Fig. 1. Characteristic larval mining in daylily leaves. Photo courtesy of Sharon Galloway.

Fig. 2. Larval leafminer. Photo by Gary J. Steck, FDACS.

(Fig. 3). Pupation takes place in the larval tunnel with spiracles protruding through the plant's epidermis (Fig. 4). Pupae (Fig. 5) are tan, about 3-4mm and usually found in the basal half of the leaf. Adult flies are stocky and shining black, often seen on daylily blooms (Fig. 6).

This insect may have two or more generations or be continuously present during the growing season, depending on region. Although even severe mining does not appear to kill plants, mining damage accumulates and will persist until leaves are removed or replaced by new growth. This means that plants remain disfigured throughout the flowering season, causing major concern for display and tour garden owners. Regulatory and trade impacts are not known as yet since this insect has only recently appeared on our radar screens.

Fig. 3. Larva in tunnel. Photo by Gaye Williams.

Fig. 5. Pupae removed from tunnels. Photo by Gaye Williams.

Fig. 4. Fly pupa in larval tunnel. Photo by Gaye Williams.

Fig. 6. Adult fly on daylily flower. Photo by V.J. Hickey, courtesy of P. Hickey.

No formal studies have been conducted to establish reliable chemical control methods.

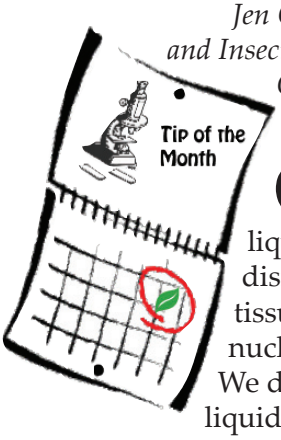
Sanitation, including removal and destruction of infested leaves may reduce potential numbers of adults. Unfortunately, control may prove difficult as infested naturalized populations of *Hemerocallis fulva*, the roadside lily, can serve as sources of reinfestation for nurseries and gardens.

At present, distribution of daylily leafminer includes Japan, Taiwan and continental U.S. (confirmed by specimens in Florida and Maryland, by the internet image from Maine; diagnosed by larval mining damage in daylily leaves in Alabama, Georgia, Louisiana, North Carolina, New York, South Carolina, Texas and Virginia). With increased awareness, this list will grow as daylily plants in other states are carefully examined. If you see leafminer damage in *Hemerocallis* leaves, please send images and information to Williams at williagl@mda.state.md.us.

Diagnostic Updates

Tip for Using Liquid Nitrogen and a Mini-BeadBeater-1 for Tissue Disruption

Jen Olson, Plant Disease and Insect Diagnostic Laboratory, Oklahoma State University

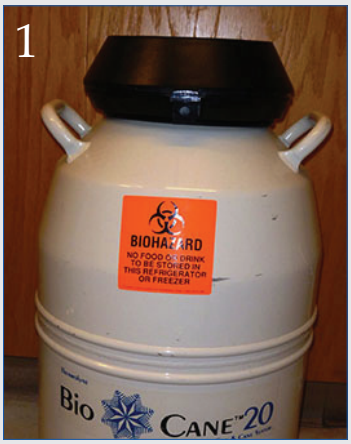


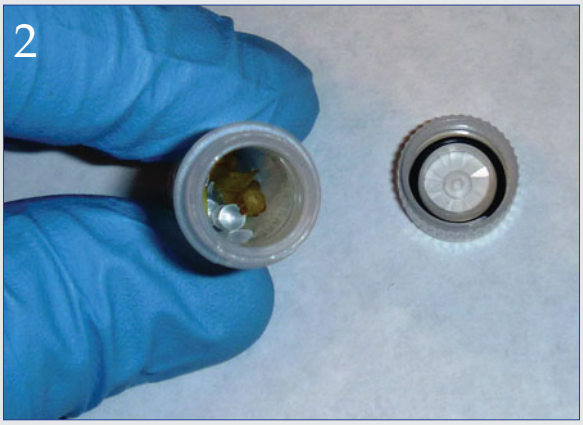
Our laboratory routinely uses liquid nitrogen to disrupt plant and insect tissues in preparation for nucleic acid extraction. We do not have access to a liquid nitrogen tank in our building so we have developed a solution to running across campus to get liquid nitrogen for DNA and RNA extractions. It is also much safer than having an open vat of liquid nitrogen on the lab bench.

Several years ago we purchased a 20L dewar for liquid nitrogen storage (Fig. 1). We fill the dewar approximately every four months with liquid nitrogen. The purchase of this tank has greatly reduced the amount of liquid nitrogen we waste and has saved us time since we no longer need to fill a small container several times a week. As an added advantage, the tank can be used for storage of a culture collection.

When we need to perform DNA or RNA extractions, we place plant or insect tissue inside a 2.0 ml plastic tube filled 25–50% with 2.5 mm glass beads (Fig. 2). It is important to try out different tubes because some plastic microcentrifuge tubes will break when immersed in liquid nitrogen. The beads may also damage the plastic during homogenization so you may need to try different size beads.

The tubes are clipped onto a "stick" that consists of three or four cryocanes squeezed together to make one long stick (Figs. 3, 4). The lid to the 20L dewar is opened and the stick is placed directly in the liquid nitrogen (Fig. 5). After 20–30 seconds, we remove the stick and unclip the frozen tube. Although you can wear





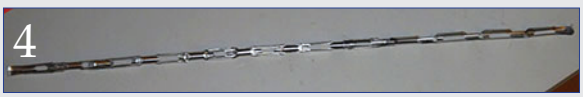


Fig. 1. 20L dewar for liquid nitrogen storage. Fig. 2. Plant tissue placed in 2.0 ml microcentrifuge tube with 2.5 mm glass beads. Fig. 3. Tube is clipped onto stick and ready for immersion in liquid nitrogen. Fig. 4. Four cryocanes are squeezed together to make one long stick.

Fig. 5. Stick and tube are immersed in liquid nitrogen directly in the dewar. Fig. 6. Frozen tube is placed in Mini-BeadBeater-1 and ready for homogenization. Fig. 7. Microcentrifuge tube containing homogenized plant tissue.

special gloves to handle the tube, we find that nitrile gloves are adequate for the brief amount of time we handle the tubes. The frozen tube is placed in the Mini-BeadBeater-1 (BioSpec Products, Inc.) and processed for 20–30 seconds at 4200–4800 rpm (Fig. 6). We generally repeat the freezing and homogenizing process once to ensure that all tissue is disrupted. If there is a lot of tissue on the lid of the tube, we will briefly centrifuge the tube to concentrate the tissue at the bottom of the tube. The sample is ready for nucleic acid extraction (Fig 7).

Check out the Summer 2011 edition of the *WPDN First Detector News* on Invasive Horticultural Plants


Western Plant Diagnostic Network
First Detector News
A Quarterly Pest Update for WPDN First Detectors
Summer 2011 edition, volume 4, number 3
NPDN
In this Issue
Page 1: Editor's Note: Invasive Horticultural Plants
Page 2-3: Introduction to Invasive Horticultural Plants
Page 4 -6: Six examples of Invasive Horticultural Plants
Dear WPDN First Detectors, Have you ever planted a plant in your garden, landscape, or commercial fields and found that it very quickly spread and took over the area? Ivies, certain bamboos, Pink jasmine, Mexican primrose, *Vinca major* and Tree of Heaven *(Ailanthus altissima)* are some of the common invasives in horticulture. Most of the plants used in gardens and landscaping do not invade or harm wildland areas. But a few vigorous species can - and do - escape from cultivation into open landscapes where they cause a variety of ecological problems. They crowd out native plants, insects and animals, clog waterways, and can lead to increased flooding, fire and crop losses. Joseph (Joe) DiTomaso, University of California Cooperative Extension specialist for weedy species, has contributed his expertise to this edition. Joe DiTomaso and Evelyn Healy have authored two excellent volumes, entitled "Weeds of California and the Other Western States," 2007, and can be purchased at ANR Catalog and Amazon. Please find links to the NPDN family of newsletters at: Newsletters
Arundo donax - Giant Reed
Ivy gone wild
Western Plant Diagnostic Network News
WPDN

Pricing Commercial Image Use

How Much Should You Charge?

Joseph LaForest, Center for Invasive Species & Ecosystem Health, University of Georgia

Photographers who have posted images in the Bugwood Image Database often receive requests to use their images in commercial projects. For the purpose of this article, I'm going to assume that the photographer owns the copyright to their images…working at a land-grant university, federal institution, or other non-profit can throw this into question (but I'll cover that in a different article).

When thinking about charging fees for commercial use of images, everyone has to answer the question "What should I charge for use of an image?" Although there are many nuances that can possibly apply to commercial licensing of images, there are four main ways that I have seen people use to determine a price:

1. Flat-Rate
2. Royalty-Free
3. Rights Managed
4. Modified Flat-Rate

Flat-Rate

This is pretty straight forward. For any request to use an image, a set fee is charged. Permission is granted for the image to be used in the project that was specified in the request only. Any other uses would be a separate request. A photographer can set their price at whatever they like. Common values I've seen from photographers in the Bugwood Image Database range between $50 and $500.

Here's an example: Gopher's Publishing (GP) requests use of an image by Sam Arnold for a billboard. Sam's flat rate is $400. After paying Sam for the image, GP decides to use the image on a circular to customers. They make a request and Sam charges $400. Five years later, GP decides to use it again as the cover of a book and an interior photo of another book. For each request, Sam charges $400. Assuming that GP decided to use the images in all cases, Sam has received $1,600 for use of the image.

Royalty Free

Despite the word FREE being in the name, the images do cost money. Royalty Free simply means that after paying money for use of the image, the buyer has permission to use the images as much as they want without paying additional royalties.

Royalty Free rates vary widely by photographer. Examples I have seen can range widely from $10 for small images (~200 pixels on the long edge) to $800 for very large images (~5,000 pixels on the long edge). Depending on the uniqueness of the images and prestige of the photographer, higher fees are not uncommon. Some photographers also place a time limit on the license.

Using the previous example: Gopher's Publishing (GP) pays Sam Arnold $400 for four year, Royalty Free license to an image that will be used on commercial projects. They still have permission to use the image in the circular since the license was Royalty Free. When they choose to use the images in the textbooks, they find that the license has expired. They contact Sam and pay an additional $400 for a new license. Over the entire use of the image, Sam has received $800 for allowing use of the image.

Rights Managed

This is a far more detailed way to license the use of images. The price charged for the images depends on how the image will be used. Since there is more detail in the licensing, there is more work required in figuring out the cost. Typical considerations for the calculation include:

- Usage type — advertising, retail, publishing/editorial, electronic, etc.

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

DIAGNOSTICS COMMITTEE

Diagnostics Committee

Anne Vitoreli, Committee Chair, University of Florida, Department of Plant Pathology

The Diagnostics Committee held a conference call on September 15, 2011, and the following agenda items were discussed:

- Protocols for NPDN lab accreditation
- SOP's in progress
- Proficiency panels
- Sentinel Plant Network regional workshops
- Diagnostics Committee poster for Nat'l Meeting

The next call is tentatively scheduled for October 13, 2011. We will cancel if there are no new agenda items to be discussed.

There will be NO November Diagnostics Subcommittee conference call.

The December Diagnostics Subcommittee conference call is scheduled for December 8, 2011.

NATIONAL DATABASE

National Database Committee

Nancy Gregory, Committee Chair, University of Delaware, Department of Plant and Soil Sciences

Following the last newsletter, the National Database Subcommittee held a conference call on September 14, 2011. The subcommittee continues to work on reviewing the extensive NPDN pest lists. The agenda included:

- Discussion of change submissions
- Sentinel Plant Network data
- Discussion of Insect pest files
- Poster for National Meeting

The next meeting will be held on October 12, 2011.

TRAINING EDUCATION

Training and Education Committee

Dick Hoenisch, Committee Chair, University of California at Davis, Department of Plant Pathology

The Training and Education Committee conducted a conference call on September 19, 2011, and the following agenda items were discussed:

- Review and update of modules
- National Meeting in Berkeley, CA
- National Meeting First Detector awards
- Web update
- Protect US update
- Sentinel Plant Network (SPN) update

The next meeting will be held on October 18, 2011.

WEBSITE COMMITTEE

Website Committee

Karen Scott, Committee Chair, Cornell University, Department of Plant Pathology and Plant–Microbe Biology

The Website Committee conducted a conference call on September 12, 2011 and the following agenda items were discussed:

- National Meeting
- Website Committee poster
- Access policy of the website

The next Website Committee conference call is scheduled for October 3, 2011.

continued from page 6 ...

- Image visibility — front cover, magazine insert, postcard, home page, etc.
- Duration of license — pretty much any length of time
- Print size — ¼ page, full page, etc.
- Circulation — how many copies will be distributed?
- Regions of use — worldwide, US only, regional, etc.
- Target audience — agriculture, food, education, etc.

As you would expect, all of these variables have wild implications for the price that is charged. Using the example above and couple of the online stock photo calculators, here is a range what GP would be looking at:

- Billboard (up to 1 year, 4'x6' size, up to 5 Locations, minor element, in the US) – $660–$2,500
- Circular (6 month campaign, ½ page, 5,000 copies, US only) – $500–$1,300
- Textbook (published for 1–2 years, full page, front cover, 2000 copies, US only) – $360–$800
- Textbook interior (published for 1–2 years , 1/4 page, 10,000 copies, US only) – $120–$290

In this pricing method, Sam would be paid anywhere from $1,640 to $4,890 for the use of the images.

Modified Flat-Rate

This model takes into account that not all image uses are the same but not in anywhere near the detail of Rights Managed licensing. Three factors are looked at:

1. Exposure rating — How prominent will the image be from 1–10 (1 for thumbnail on a small website, 10 for Super Bowl commercials)
2. Profit rating — How much does the user intent to profit from using the image for 1–10 (1 being a small ad in non-profit trade magazine, 10 being luxury cars)
3. Base price — The most you would ever charge for your image (e.g. selling luxury cars in the Super Bowl). This essentially would be your flat rate which will be adjusted based on Exposure and Profit of the use using the formula below.

Usage Fee = (Exposure Rating x Profit Rating) % x Base Price

Applying this to the example:

- Billboard—*local use for Ag Expo*
  (3 x 4)% x $2,500 = 12% x $2,500 = $300
- Circular—*regional use for new herbicide*
  (4 x 6)% x $2,500 = 24% x $2,500 = $600
- Textbook—*national use for biology*
  (5 x 3)% x $2,500 = 15% x $2,500 = $375
- Textbook—*national use for biology*
  (3 x 3)% x $2,500 = 9% x $2,500 = $225

Judgments on the profitability and exposure will vary from person to person but this method does allow for taking that into account. By this method, Sam would have received $1,500 for the use of his image.

As you can see, the different pricing methods vary greatly in the revenue that may potentially be generated from commercial use. This does not take into account whether or not the person requesting use of the images chooses to pay what you ask. It is possible that more people would pay for use of an image through the royalty free system rather than dealing with the details and higher price of a rights managed license. This also does not take into account any fees that may be incurred if an individual uses an agency or service to process the request and licensing. Despite these limits, hopefully this quick primer on different options will help in finding a model that works for you.

Security Tip: Facebook

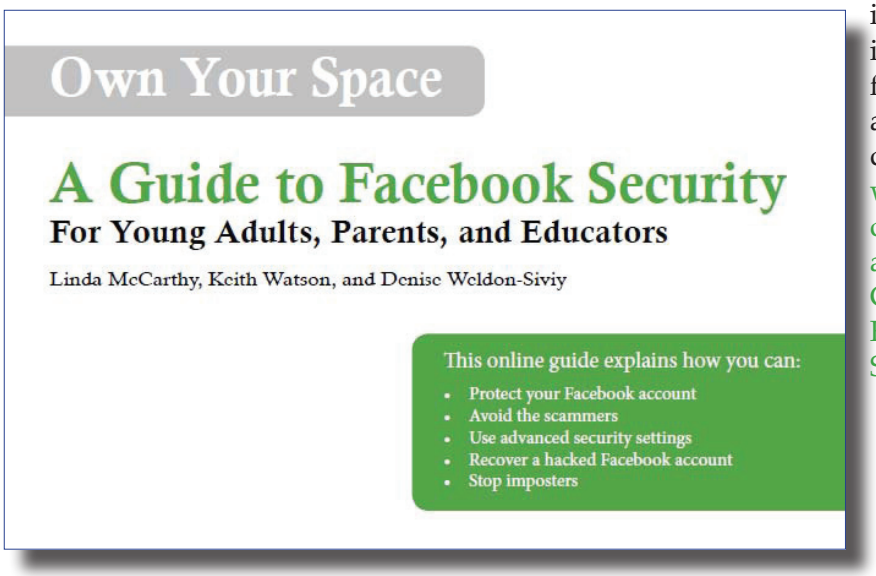
Understanding It So You Can Secure It

Mike Hill and Eileen Luke, CERIS, Purdue University

Keith Watson, a Research Engineer with CERIAS (Center for Education and Research in Information Assurance and Security) at Purdue University has been collaborating with the NPDN for over seven years, providing security assessments, reviews, training, presentations and general consulting. His expertise has been invaluable to the network in aiding IT staff in achieving the number one goal of operating and maintaining good security practices.

As technology and social media evolve, new demands arise in securing information. Facebook, a popular social networking service, originally was a means for individuals to link socially online and share their information on a wider scale. Companies, institutions, and organizations have seen the value of such a tool and have created a presence on Facebook as well. As is the case with all software tools available on the internet, good security practices are important for both the user and the provider.

Keith Watson, along with Linda McCarthy and Denise Weldon-Siviy, wrote an article titled, 'A Guide to Facebook Security.' This guide explains how you can protect your Facebook account, avoid the scammer, use advanced security settings, recover a hacked Facebook account, and stop imposters. Take the time to read this article; it's worth it. This document is available for free on Facebook and can be downloaded at www.facebook.com/safety/attachment/Guide%20to%20Facebook%20Security.pdf



Training and Education

Sentinel Plant Network Regional Workshop at the Arnold Arboretum

Rachel McCarthy, Department of Plant Pathology and Plant-Microbe Biology, Cornell University

The Sentinel Plant Network (SPN) held its first workshop for northeast regional gardens at the Arnold Arboretum in Boston, MA, on September 22–23. This was the first workshop in a series intended to engage public gardens in the early detection of regionally significant high-consequence pathogens and insect pests.

The Sentinel Plant Network, a cooperative endeavor between the American Public Gardens Association (APGA) and the NPDN, expects to enhance diagnostic capabilities via improved communication between public gardens and expert diagnostic facilities. The workshop introduced garden professionals to the NPDN and its mission with presentations and hands-on activities on how to use our online resources, how to collect and submit samples, and where to find information on their state's diagnostic laboratory so they can send in suspect samples.

Photo top, George Hudler, and bottom, Rich Cowles, talking to workshop participants at the Arnold Arboretum. Photos courtesy of Dan Stern, American Public Gardens Association.

The program also featured the unveiling of the first three Train-the-Trainer modules designed specifically for the SPN—Module 1: Public Gardens and the Sentinel Plant Network, Module 2: Photography for Diagnosis, and Module 3: Submitting Samples to the National Plant Diagnostic Network. Of course the most talked about sessions were the pest walks led by George Hudler, Director of the NEPDN, and Rich Cowles from the Connecticut Agricultural Experiment Station, who in addition to highlighting specific insects and plant diseases, focused time on improving scouting by sharpening one's observation skills.

The meeting was well attended with 28 people from 18 gardens and professionals from horticulture and IPM departments, as well as, education and outreach departments. Participating gardens were from Massachusetts, New York, Pennsylvania, Connecticut, Maryland, Maine and New Jersey.

The next workshop is intended for midwestern gardens and is scheduled for October 17–18 at the Morton Arboretum in Lisle, IL. The western regional workshop is scheduled for December 5–6 at the Rancho Santa Ana Botanic Garden in Claremont, CA. Additional workshops are planned in early 2012 for gardens in the southern and Great Plains regions.

There may be funds available for NPDN diagnosticians and entomologists to attend as guests; those interested should contact Rachel McCarthy at rachel.mccarthy@cornell.edu.

Regional News

Soybean Vein Necrosis Virus Confirmed in New York

Gary Bergstrom, Department of Plant Pathology and Plant-Microbe Biology, Cornell University

The presence of a new soybean disease was confirmed this week in New York. The pathogen is Soybean vein necrosis virus (SVNV). It was first described in Tennessee in 2008, and in Arkansas, Illinois, Kansas, Kentucky, and Missouri in 2009. Dr. Don Hershman of the University of Kentucky commented recently that the disease was observed in late season at low levels in Kentucky many years before its viral etiology was proven. The symptomatic plants in New York came from a soybean field in Ontario County. Symptoms observed in the field were vein-clearing followed by 'scalded' reddish areas around the veins and a browning of the veins, especially on the lower leaf surface (see the photo). Dr. Ioannis Tzanetakis of the University of Arkansas, a leading researcher on SVNV, positively identified RNA of SVNV in samples from Ontario County. I credit the timely diagnosis of SVNV in New York to excellent communication among Northeastern Plant Diagnostic Network participants. An NEPDN listserve communication from Nancy Gregory at the University of Delaware with photos of SVN symptoms observed in Delaware arrived at the precise time that I was trying to diagnosis unusual symptoms on soybean in New York. Recently SVNV was confirmed in Delaware, Maryland, and Virginia, with pending diagnoses in Pennsylvania. Yesterday I viewed photographs with symptoms from a field in Herkimer County, NY that also appear to be caused by SVNV; the symptoms were predominantly on the upper, youngest leaves and were being described as a 'top dieback'.

I credit the timely diagnosis of SVNV in New York to excellent communication among Northeastern Plant Diagnostic Network participants.

What is known about SVNV? The answer is not very much. It is thought to be transmitted from soybean to soybean by thrips (soybean thrips and perhaps others). Soybean thrips are observed in New York along with a number of other thrips species. The virus has been placed in the Tospovirus group of plant RNA viruses (stands for Tomato spotted wilt virus) which are transmitted by thrips. Finding it this year doesn't mean we will find it next year. We don't know if the virus can also be transmitted through seed or by any other means. We don't know if it will have an impact on yield. Potentially the virus may be transported long distances by thrips in one growing season or the virus may survive locally in weed hosts (no one has demonstrated this yet) and then be transmitted locally by thrips when their population increases within a season. Tospoviruses are circulative/propagative in their association with thrips, so if thrips survive our winters, SVNV might well

Photo courtesy of Gary C. Bergstrom, Cornell University.

survive in the living thrips. Insecticidal seed treatments may have a role to play in killing thrips on young soybean plants and reducing the incidence of early virus infection. Resistant varieties appear to be the main path to sustainable management and several investigators are assessing varieties in other parts of the country. There is no basis to recommend that farmers do anything substantially different now in their cropping practices just because we found this new virus. For now, I would classify its presence in NY only as a potential problem worth keeping an eye on. We encourage growers and consultants in New York to inspect any still-green soybean plants for possible SVNV and to inform their local Cornell Cooperative Extension field crops educator if they find symptoms that are similar to soybean vein necrosis.

Upcoming Events

National Events

November 6–9, 2011
NPDN National Meeting
Berkeley, CA

November 13–16, 2011
ESA 59th Annual Meeting
Reno, NV

Rachel McCarthy, Editor
NEPDN
Cornell University