

## *Phytophthora ramorum* Sample Routing

Joel Floyd, Domestic Diagnostics  
Coordinator, USDA-APHIS-PPQ National  
Identification Services

Recent changes in the *Phytophthora ramorum* PASS (Potential Actionable Suspect Sample) policy and a need to screen symptomatic samples prior to sending them to USDA-APHIS, Plant Protection and Quarantine labs for confirmation, has brought to light some changes and guidance that may be needed. This may be necessary for coming Farm Bill funded surveys or in the event of trace forward investigations from *P. ramorum* positive nurseries when plants have been sent to other states.

Screening of symptomatic survey samples should be handled by the state of origin when possible and if not, because of limited capacity or ability to do ELISA and DNA extraction; contact the NPDN hub laboratory for possible assistance.

The same is true in the event of a trace forward shipment arriving in a state. Regulatory officials in each state will receive the destination trace forward localities with the numbers of shipments/plants, and inspectors will do visual inspections looking for symptomatic plants for all the host species in those shipments. If symptomatic plants are encountered, we are encouraging all destination states to use their NPDN (state agriculture laboratory and/or university land grant) to process the raw plant samples. This includes ELISA testing for *Phytophthora* sp. and DNA extraction for samples with positive

ELISA results. NPDN labs with CPHST accreditation and current proficiency approval, can perform the PCR and report negatives for *P. ramorum* back to the submitter.

Unfortunately, there is no additional funding available for the individual NPDN laboratories to process these samples. NPDN laboratories that receive samples from these trace forwards can handle them in the following two ways...

If you have the ability, personnel and funding to process the samples in your laboratory, follow procedure 1.

1. NPDN laboratories that have extracted DNA from positive ELISA samples, or DNA from PCR tests at accredited/proficiency approved labs, should forward the DNA with completed PPQ form 391's and prior e-mail notification, to one of the PPQ Regional Laboratories listed in their area (page 2). As usual, please be sure to notify your SPHD and SPRO when forwarding the

### Issue Highlights

- NPDN impact survey
- Star-D meeting at UFPDC
- Diagnostic tip: testing for rose rosette disease
- IT news: first submissions
- In *Regional News*: EAB found in CT and HWA First Detector success story in VT

samples, and include in the e-mail notification the number of samples being sent and the overnight carrier tracking number. According to the recently revised **PASS policy for *P. ramorum***, these two PPQ laboratories can now perform final confirmations.

If you **DO NOT** have the ability, personnel and funding to process the samples yourself, follow procedure 2.

2. For NPND laboratories receiving trace forward samples that cannot perform ELISA and/or DNA extractions, contact the hub lab in your area to determine if they can provide you with assistance. *If they cannot, the Cornell University and University of Florida hub laboratories are available to provide these services.*

*Any region can send their trace forward samples to Cornell or Florida!*

Any region can send their trace forward samples to Cornell or Florida! Please be sure to send an e-mail notification with the number of samples being sent and the overnight carrier tracking number when submitting the samples. The Cornell University and University of Florida hub laboratories will conduct the testing needed and forward the DNA with completed PPQ form 391's and prior e-mail notification, to one of the PPQ Regional Laboratories listed below. The contact information for the NPND laboratories is provided here:

**Northeast Region:**

Karen L. Snover-Clift  
Cornell University  
Plant Disease Diagnostic Clinic  
334 Plant Science Building  
Ithaca, NY 14853  
(607) 255-7850

**Southern Region:**

Anne Vitoreli  
Plant Disease Clinic  
UF Bldg 78 Mowry Road  
P.O. Box 110830  
Gainesville, FL 32611-0830  
(352) 392-1795  
(352) 392-3631 Ext. 254  
(Carrie Harmon)

For questions about individual states, contact your SPRO first, and if further guidance is needed, the SPHD can contact the PPQ *P. ramorum* regional program managers, PPQ Eastern Region, Anthony Man-Son-Hing at phone: (919) 855-7331 (office), e-mail: [anthony.man-son-hing@aphis.usda.gov](mailto:anthony.man-son-hing@aphis.usda.gov) or in the PPQ Western Region, Stacy Scott, at phone: (970) 494-7577, e-mail: [stacy.e.scott@aphis.usda.gov](mailto:stacy.e.scott@aphis.usda.gov).

**PPQ Eastern Regional Laboratory:**

Grace O'Keefe  
Plant Pathologist Identifier  
USDA, APHIS, PPQ  
105 Buckhout Lab  
Penn State University  
University Park, PA 16802  
[grace.okeefe@aphis.usda.gov](mailto:grace.okeefe@aphis.usda.gov)  
Lab: (814) 865-9896  
Cell: (814) 450-7186

**PPQ Western Regional Laboratory:**

Craig A. Webb, Ph.D.  
USDA, APHIS, PPQ  
Department of Plant Pathology  
Kansas State University  
4024 Throckmorton Plant Sciences  
Manhattan, KS 66506-5502  
[craig.a.webb@aphis.usda.gov](mailto:craig.a.webb@aphis.usda.gov)  
Cell (785) 633-9117  
Office (785) 532-1349 🌿

## NPDN Impact Survey

Mary E. Burrows, Department of Plant Sciences and Plant Pathology, Montana State University

In order to provide our administration impact data to support continued funding of the NPDN, several states have participated in an impact survey for their lab. The main impediment for many states to participate was applying to their Institutional Review Boards for Human Subjects approval. This is required if we want to publish the survey. If you would like to participate, but are not comfortable with the IRB process, you can still participate and we will not publish your data. We currently have data from seven states. The economic impact of diagnoses by those laboratories in 2011 was approximately \$19 million, a 1:69 return on NPDN investment in the lab.

The majority of questions is designed for you to get feedback from your clients, and will be helpful for your year-end reporting. The process is very simple and the survey is provided for you. You can modify the questions. We will set up the survey online for you with a unique link which you can send to your clients, county extension offices, etc.

If you are interested in participating, please contact Mary Burrows ([mburrows@montana.edu](mailto:mburrows@montana.edu); (406) 994-7766). 🌱

## NPDN STAR-D Auditor Checklist Development Meeting at the UF Plant Disease Clinic

Dawn Dailey O'Brien, National Quality Coordinator, NEPDN, Cornell University and Karen Snover-Clift, National Quality Manager, NEPDN, Cornell University

On June 19–21, 2012 the STAR-D team consisting of Karen Snover-Clift, Dawn

Dailey O'Brien, and their colleagues Pat Shiel, Kathy Burch and Geoff Dennis from USDA-APHIS-PPQ-CPHST met to continue the work on the STAR-D project. Once again, Anne Vitoreli opened her laboratory (UF Plant Disease Clinic-UFPDC) at the University of Florida in Gainesville, FL to be the stage of a practice audit, much as she had in the fall of 2011 as part of the auditor training course.

The main focus of the three-day gathering was to create audit checklist templates to provide assistance to auditors during the audit process. The group methodically reviewed the UFPDC's Quality Manual and from that generated appropriate questions to address each and every section of the manual. The questions included in the checklists were designed to gather objective evidence during an audit.

These checklist templates will serve as guides to the auditors. Although the templates were based on UFPDC's Quality Manual they are completely customizable so an auditor can add questions relative to a particular lab and most NPDN labs will likely have a Quality Manual very similar to UFPDC. Instead of having auditors start from scratch at each audit the templates will



(Top) Kathy Burch asks Anne Vitorelli a question about her equipment calibration while Geoff Dennis looks on during the audit at UFPDC. (Bottom) Anne Vitorelli explains the sample sign in process to Pat Shiel, Geoff Dennis and Karen Snover-Clift during the audit at UFPDC. Photos courtesy of Dawn Dailey O'Brien, Cornell University.



give them an excellent starting point. As with the quality manual and associated documents, our goal with the checklist templates is to minimize the time and effort an individual needs to spend developing documents. We understand the value of our diagnostician's time and developing all the STAR-D templates will reduce duplicative efforts.

In general, using a checklist ensures that the audit at a minimum will address the requirements to the organization's management system. In addition, checklists help to ensure that an audit is conducted in a systematic and comprehensive manner. After the checklist templates were created the

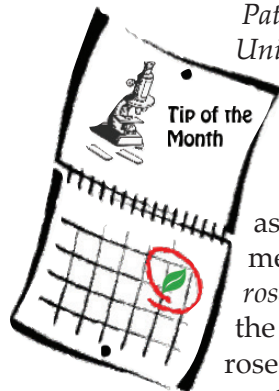
group performed a gap audit at the UFPDC successfully implementing the newly created checklists.

The focus of the STAR-D group this upcoming year will be preparing the trained auditors from the fall 2011 workshop to gain practice in real-world laboratory audits during four scheduled practice audits in NPDN laboratories around the country. Additionally, Quality Management and Auditor Workshops are in the planning stages. None of these activities would be possible without the support of our CPHST partners and financial support from the 2012 Farm Bill. 🌱

## Diagnostic Updates

### How We Test for Rose Rosette in Oklahoma

Jen Olson, Department of Entomology and Plant Pathology, Oklahoma State University



We have had many colleagues ask us to share our method of diagnosing *rose rosette virus* (RRV), the proposed cause of rose rosette disease. We are still working out some of the kinks, but are willing to share our technique. At this point, the test does not *always* work, but we do have good results most of the time. Overall, the results of this assay correlate with our visual diagnosis. This is the method that we use at the Plant Disease and Insect Diagnostic Laboratory at Oklahoma State University. It is likely that you will have to make some adjustments if you choose to use this testing method in your laboratory.

The sample material required for RRV

testing is symptomatic shoots. It is best if clients clip at least 6 inch shoots and place them in a sealed plastic bag. If the



Figure 1. Ornamental rose with symptoms of rose rosette disease. Photo courtesy of Oklahoma Cooperative Extension Service (OCES).

rose leaves are damp, they should wrap the shoots in a dry paper towel. The plant tissues selected for RNA extraction are the symptomatic leaves and/or floral parts (Figure 1).

The method we use is based on the procedure developed by Laney *et al* (1). In this paper, the extraction method calls for a large volume of plant material and a high speed centrifuge. In some cases, we are limited on the quantity of infected plant tissue and this equipment is not readily available in our laboratory. As a result, we decided to try the RNeasy Plant Tissue Kit from Qiagen, Inc. We follow the manufacturer's instructions and we generally use 70 mg of plant tissue for the assay. To date, this method has worked quite well for detection of RRV in our diagnostic laboratory.

Following RNA extraction, we generate cDNA by preparing the master mix shown in Table 1. According to the instructions that are included with the reverse transcriptase enzyme used in our laboratory, following sample preparation, the tubes should be incubated at 37°C for 1 hour. If you use another type of reverse transcriptase, you will need to check the temperature and time requirements for your enzyme. It is expected that other reverse transcriptase enzymes would work well, but these adjustments will need to be made.

For the PCR master mix, we use a 2X ready mix. We have not tried this test with "homemade" master mix because we no longer stock these products for

most of our diagnostic testing. We utilize student labor and find that we have fewer mistakes when we use a ready mix as opposed to a homemade master mix where many more ingredients are required. This is our experience and yours may be different.

When we test for RRV, we actually prepare two different master mixes. The first master mix is to test for RRV and uses primers from Laney *et al.* (1). The second master mix uses primers that detect the large subunit of ribulose biphosphate carboxylase oxygenase (RubiscoL) (2). This compound is found in all plant cells and we use this test to confirm that our RNA extraction was adequate and there are not too many inhibitors in the PCR reaction. We have had cases where we expected a positive for RRV and the PCR test was negative. When we prepared a fresh RNA extraction using less plant material (50 mg), we obtained positive test results for both the RRV and RubiscoL PCR. We suspect that this problem is due to a large amount of inhibitors in the rose plant tissue. By using less plant tissue or diluting the cDNA, you can dilute the inhibitors and lessen their interference with the reaction. We generally do not dilute our cDNA and add it directly to the PCR master mix.

At this point, we have not tried a

**Table 1. Preparation of master mix for cDNA synthesis**

Reagent	Vol. for 1 Reaction (ul)	___ reactions*	Final Conc.
RNase free water	7.0		
5X buffer	5.0		1X
dNTP mix (2 mM stock)	5.0		400 uM
Random hexamers (5 uM)	2.5		0.5 uM
Reverse transcriptase (200 U/ul)	0.5		100 units
Aliquot volume	20.0		
Template RNA	5.0		

*\*Multiply by the number of tubes needed plus 1-2 (10-15%) more than needed to allow for waste while pipetting.*

multiplex where we mix the RRV and RubiscoL primers in one tube. Instead, each master mix is placed in a separate tube. The PCR mix and primer sequences are shown in Tables 2 and 3 respectively. It is satisfactory to put the RRV and RubiscoL tubes into the same thermal cycler machine and use the same cycling parameters. The primer sets have only a 1°C difference in annealing temperature,

amount of bovine serum albumin (BSA) to our reactions and this seems to help with the inhibition problem. If you attempt to follow this procedure, we suggest that you include this compound.

For those diagnosticians who are interested in having samples tested for RRV, but do not wish to run this procedure, we are willing to accept

**Table 2. Preparation of master mix for PCR**

Reagent	Vol. for 1 Reaction (ul)	___ reactions*	Final Conc.
2X ready mix (we use Econotaq Plux Green 2X Master Mix by Lucigen Corp)	12.5		1X
PCR grade water	4.75		
Forward primer **(5 or 2 uM)	2.5		0.5 or 0.2 uM
Reverse primer (5 or 2 uM)	2.5		0.5 or 1.2 uM
BSA (10 mg/ml)	0.25		0.1 mg/ml
Aliquot volume	22.5		
Template cDNA	2.5		

\*Multiply by the number of tubes needed plus 1-2 (10-15%) more than needed to allow for waste while pipetting.

\*\*Primers for RRV are used at 5 uM stock and 0.5 uM final concentration. Primers for RubiscoL are used at 2 uM stock and 0.2 uM final concentration.

**Table 3. Primers pairs and expected band size for RRV testing**

Primer name	Primer Sequence	Expected band size	Source
RRV for	CAGAATGAACCATAGATGTC	319	Laney et al.
RRV rev	AATGGTCTGCTCGAGATT		
RbcL-C705	CATCATCTTTGGTAAAATCAAGTCCA	171	Nassuth et al.
RbcL-H535	CTTCCAAGGCCCGCCTCA		

so we have used the lower temperature of 53°C. The cycling parameters used by our laboratory are 94°C for 2 min, 35 cycles of 94°C for 30s, 53°C for 10s, and 72° for 10s, with a final extension at 72°C for 10 minutes. Following amplification, we visualize our PCR products on a 1.5% agarose gel.

When we first started running this test, we felt that we were running into inhibition problems that interfered with the PCR. We have since added a small

amount of bovine serum albumin (BSA) to our reactions and this seems to help with the inhibition problem. If you attempt to follow this procedure, we suggest that you include this compound. For those diagnosticians who are interested in having samples tested for RRV, but do not wish to run this procedure, we are willing to accept samples for RRV diagnostic testing from out-of-state. Please contact the PDIDL in advance so that we can send you our PPQ526 permit that includes instructions for properly packaging samples. Our prices are subject to change, but our current fee is \$25 per sample for RRV testing. We do require prepayment for testing of out-of-state samples and checks should be made out to Oklahoma State University.

We encourage others to try this method in your laboratories and let us know how it works. If you have questions or comments, please contact [jen.olson@okstate.edu](mailto:jen.olson@okstate.edu) or (405) 744-9961.

1. Laney, A. G., Keller, K. E., Martin, R. R. and Tzanetakis, I. E. 2011. A discovery 70 years in the making: characterization of the Rose rosette virus. *J. Gen. Virology*. 92:1727-1732.
2. Nassuth, A., Pollari, E., Helmeczy, K., Stewart, S., and Kofalvi, S. 2000. Improved RNA extraction and one-tube RT-PCR assay for simultaneous detection of control plant RNA plus several viruses in plant extracts. *J. Vir Meth.* 90:37-49. 🌱

## Workshop: Nucleic Acid-based Pathogen Detection

*Paul Vincelli, Department of Plant Pathology, University of Kentucky*

A hands-on workshop for applied plant pathologists on nucleic acid-based pathogen detection will be held at the University of Kentucky in Lexington. The workshop will begin on Tuesday, January 22, 2013, with introductory lectures and lab activities suited for those with little PCR experience. All participants—beginners and experienced alike—will attend from Wednesday morning, January 23, 2013, through mid-day Friday, January 25, 2013, during which time participants will design, execute, and interpret three real-time PCR experiments (SYBR® Green and Taqman® assays). Presentations and discussions will include basic theory of real-time PCR, experimental controls, PCR inhibition, use of PCR kits, verifying amplicon identity, arrays, minimizing contamination, troubleshooting, sequencing (direct vs. cloning), and selecting fluorophores. Activities and discussions will be included on primer

design, interpreting BLAST searches and the use of curated genomics databases. The topic of quantification will be covered but not in depth. Registration will be \$250 and \$300 for Wednesday-Friday and Tuesday-Friday, respectively. For more information, contact Paul Vincelli ([pvincell@uky.edu](mailto:pvincell@uky.edu)). 🌱

Don't miss...the  
APSnet Feature:



*Healthy Plants • Healthy World*

## The Plant Diagnostic Lab Experience

Linnea G. Skoglund, Plant Disease Diagnostician, Montana State University  
and Tamla Blunt, Plant Disease Diagnostician, Colorado State University



# IT News



## National Repository CERIS

### Regional Access

Mike Hill and Eileen Luke,  
CERIS, Purdue University

Over the last month regional access has been opened up to several users across the NPDN network. This level of access will provide users the ability to view reports, maps, and charts for their entire region.

One of the biggest benefits of regional access is the daily first submission by state e-mail report.

the first time that pest/pathogen was uploaded to NPDN as confirmed for that state. This report will include data uploaded by the diagnostic labs within the region and occasionally may include a state from outside of that region. This event occurs when a lab within the region diagnoses a sample for a state outside of the region. You can verify which lab uploaded the data by visiting the online report at: [https://npdn.ceris.purdue.edu/htbin/npdn\\_h1stocc.com](https://npdn.ceris.purdue.edu/htbin/npdn_h1stocc.com) (username and password required). Data contained within these daily e-mail reports along with all data in the National Repository should be treated as

The screenshot shows a Mozilla Firefox browser window titled "NPDN National Database - Mozilla Firefox". The address bar shows the URL [https://npdndev.ceris.purdue.edu/htbin/npdn\\_firststocc.com](https://npdndev.ceris.purdue.edu/htbin/npdn_firststocc.com). The page content is titled "First Submission by State Report".

**Date of Search:** 12/16/10  
**Time of Search:** 02:36 PM EST  
**Sorted By:** Sample Date (Descending)  
**Number of Records:** 1

**Search Criteria**  
**Submission Date:** 12/16/2010-12/16/2010  
**State(s):** IN  
**Diag. Lab:** 9995

**\*Note:** Report contains records of pests, weeds, or diseases submitted to the NPDN Repository for the first time in that state for that pest code. Please note *Phytophthora sp./spp.* will be a different code than *Phytophthora parasitica* and each will have its own "first submission" for a state record. It is NPDN Policy that records submitted to the NPDN repository are final results, not presumptive positives. It also is NPDN policy that diagnoses of regulatory significance are not to be submitted to the NPDN repository until after the SPRO and SPHD have been notified of the diagnostic result.

Submission Date	Sample Date	State	Diagnostic Lab	Pest	Host Common Name
12/16/2010	12/16/2010	IN	9995 (IN)	Not On List [Not On List ]	Unknown; No Site Specified

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This e-mail report represents pest/pathogens by state that have been uploaded to the National Repository as confirmed for the first time. It is important to recognize that these first submissions may not necessarily indicate the first time that pest/pathogen has occurred in the state, but rather indicates

confidential and not be distributed.

Additional information on this report can be found in the [January 2011 newsletter](#). If you have any questions on regional access or on the first submissions by state report, please contact Mike Hill (765) 494-9854 or Eileen Luke at (765) 494-6613. 🌿



## Aisal Brown Joins PDIS

GPDN is pleased to announce that we have hired Aisal Brown as our newest PDIS team member! Aisal began working June 25. Aisal's background is in computer science with web development, database programming, application development and end-user support. Join us to welcome Aisal to the team!!



Aisal and Judy can be reached at [pdis@ksu.edu](mailto:pdis@ksu.edu).



## Emerald Ash Borer Found Connecticut

On July 20, 2012, the Connecticut Agricultural Experiment Station (CAES) and the Department of Energy and Environmental Protection (DEEP) announced that the emerald ash borer (*Agrilus planipennis*) was detected in Prospect, CT on July 16, 2012 by staff members at CAES. The identification has been confirmed by federal regulatory officials in the USDA Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ). This is the first record of this pest in Connecticut, which is added to 15 other states where infestations have been detected. Ash makes up about 4% to 15% of Connecticut's forests and is a common urban tree.

The insect specimens were recovered in Prospect from a ground-nesting, native wasp (*Cerceris fumipennis*), which hunts beetles in the family

## Regional News



Purple trap used to lure EAB. Photo courtesy of Dawn Dailey O'Brien, Cornell University, Bugwood.org.

Buprestidae, including the emerald ash borer. The wasp provides a highly efficient and effective “bio-surveillance” survey tool and does not sting people or pets. This work was supported by the US Forest Service. In addition, 541 purple prism detection traps, containing a special chemical lure, have been set across the state in all eight counties by the University of Connecticut Cooperative Extension System via an agreement with the USDA-APHIS-PPQ. Three additional EAB have been captured in a trap located in Prospect, while other beetles were captured in a trap in Naugatuck.

Visit [CT Department of Energy and Environmental Protection](#) and click on Emerald Ash Borer under current topics to learn more and read the full announcement. 🌿

FD Success Story  
in VT

### **Hemlock Woolly Adelgid Found by VT Forest Pest First Detector**

The hemlock woolly adelgid (HWA) has been detected, for the first time, in Bennington County, VT. A volunteer Forest Pest First Detector found infested trees on municipal land in the town of Pownal. The insect is thought to have spread there naturally from nearby Massachusetts.

HWA were first discovered in southeastern Vermont forests in 2007. Until now, known infestations of the insect in Vermont have been restricted to Windham County, covering nine towns. The Pownal find is the most recent in a series of new detections in 2012. “Our monitoring studies indicate that the warm winter weather increased survival of this damaging pest”, says Jim Esden, Forest Protection Forester with the

Vermont Department of Forests, Parks and Recreation.

The Forest Pest First Detector program trains volunteer leaders in communities throughout Vermont to increase the public’s awareness about the tree pests, assist government partners in responding to inquiries about suspect bugs, and help their communities prepare for and respond to a pest infestation. Michael Rosenthal, a retired high school teacher from New Jersey and amateur botanist, is the trained volunteer who found the adelgid in Bennington County. He took up the call to action because he has a strong interest in the natural environment. “I’ve seen the damage from the hemlock woolly adelgid when I lived in NJ. I would like to do what I can to help protect Vermont’s natural communities. I have a good knowledge of the land in my locality. I hike throughout the area several times a week.”

Visit <http://www.vermont.gov/portal/government/article.php?news=3705> to read the full announcement with the Vermont Agency of Natural Resources. 🌿



# USDA Urges the Public to Report Asian Longhorned Beetle Sightings

APHIS Newsroom

APHIS is asking the public to be on the lookout for the Asian longhorned beetle (ALB). August is a time of peak emergence for the devastating invasive pest and a critical time for building the public's awareness of ALB.

"The public is our first line of defense because early detection is crucial and could mean more trees saved," said Rebecca Blue, Deputy Under Secretary of the USDA-APHIS.

Adult beetles are most active during the summer and early fall. They can be seen on trees, branches, walls, outdoor furniture, cars, and sidewalks and caught in pool filters. With these unique characteristics, the beetle can be easy to see:

- 1 to 1 ½ inches in length
- Long antennae banded in black and white (longer than the insect's body)
- Shiny, jet black body with random white spots
- Six legs, may appear bluish in color

In addition to looking for the beetle, you can search for signs of infestation, including:

- Dime-sized (1/4" or larger), perfectly round exit holes in the tree
- Oval depressions on the bark where the eggs are laid
- Sawdust-like materials, called frass, on the ground and the branches
- Sap seeping from wounds in the tree

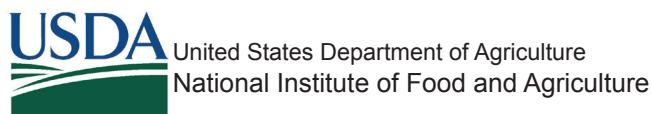


## National Events

August 4–8, 2012  
2012 APS Annual Meeting  
Providence, RI

November 11–14, 2012  
Entomology 2012, ESA 60<sup>th</sup> Annual Meeting  
Knoxville, TN

## Upcoming Events



Rachel McCarthy, Editor  
NEPDN  
Cornell University

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