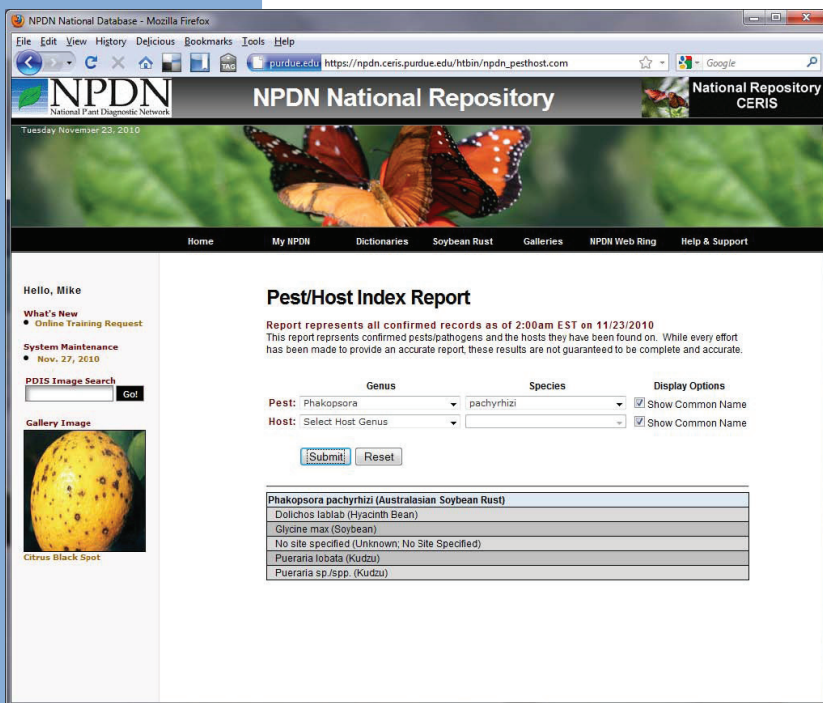


Enhancements to the National Repository

Mike Hill and Eileen Luke, Purdue University, CERIS

At the NPDN IT/Diagnosticians' joint workgroup meeting in October three new proposed enhancements were presented. The first enhancement

reported in a state to the NPDN National repository. Currently the system emails six different reports depending upon role access to regional and national program leader staff daily based on the previous day's activities. An online report feature is also available for past dates. The enhancement will provide a similar report to the SPRO's and diagnosticians with their specific state access. The enhancement will show all diagnoses from your state and all diagnoses your laboratory processed regardless of state of origin. The first release of this enhancement, with online report capability, will be at the end of 2010 and the email feature will be implemented in the first quarter of 2011.



The screenshot shows a web browser window displaying the NPDN National Repository interface. The page title is "NPDN National Repository" and the URL is "https://npdn.ceris.purdue.edu/htbin/npdn_pesthost.com". The main content area is titled "Pest/Host Index Report" and shows a search form with "Phakopsora" selected for Genus and "pachyrhizi" for Species. Below the form is a table of results:

Genus	Species	Display Options
Phakopsora	pachyrhizi	<input checked="" type="checkbox"/> Show Common Name
Host: Select Host Genus <input type="checkbox"/> Show Common Name		
<input type="button" value="Submit"/> <input type="button" value="Reset"/>		
Phakopsora pachyrhizi (Australasian Soybean Rust)		
Dolichos lablab (Hyacinth Bean)		
Glycine max (Soybean)		
No site specified (Unknown; No Site Specified)		
Pueraria lobata (Kudzu)		
Pueraria sp./spp. (Kudzu)		

The second enhancement is the pest grouping feature. This item will support grouping by species, entries without common names, and a better means to address synonyms. The pest/pathogen dictionary will still be available in its classic form and no software changes will be required for data entry on the part of the lab management systems. Most of the software for this effort has been developed, but assistance from a subgroup of the Database Program Area Committee is required to accurately determine the list of unique scientific names and to do beta testing prior to its

is an expansion of the first submitter by state report. This report represents the first time a pest/pathogen has been

Issue Highlights:

- *Duponchelia fovealis*
- Bacterial canker of kiwifruit
- Diagnostic tip: loop-mediated isothermal amplification

- NPPLAP's *P. ramorum* proficiency panel
- PDIS - new features
- In *Regional News*: red palm weevil, brown marmorated stink bug and peach fruit fly




National Institute of Food and Agriculture

targeted implementation date of March 31, 2011.

The final feature is the Pest/Host index report which shows confirmed pest/pathogens and the

hosts they have been found on. This data index is presented as a diagnostic tool to NPDN member laboratories only with a requirement to check the agreement box to maintain confidentiality of the data within the NPDN community and appropriate state plant regulatory officials. The report is able to search by both pest and host to both the genus and species level. No location or time of occurrence are provided thus not breaching confidentiality issues while still providing useful information to the diagnosticians. This feature will be implemented at the end of 2010.


Questions about these new features or any other items about the National Repository should be sent to Mike Hill mhill@ceris.purdue.edu or Eileen Luke lukee@purdue.edu. 

Thirteen New State Detections of *Duponchelia fovealis*

On May 18, 2010, the Canadian Food Inspection Agency (CFIA) notified APHIS about an interception of *Duponchelia fovealis* larvae in a nursery shipment from San Diego County, California. On July 16, 2010, APHIS confirmed a detection at a greenhouse in Vista, California. Trace-forward information provided by the affected nursery resulted in the expansion of detection surveys in 26 counties within California.

During the months of September and October, APHIS and State Departments of Agriculture have confirmed the presence of *Duponchelia fovealis* in portions of twelve additional states including; Alabama, Arizona, Colorado,

Florida, Georgia, Mississippi, North Carolina, Oklahoma, Oregon, South Carolina, Texas, and Washington.


A type of moth, *Duponchelia fovealis* originated in southern Europe and North Africa and has a wide range of host plants, including peppers, pomegranates, aquatic plants, bedding plants and nursery stock. [Read the full SPRO letter here.](#) 

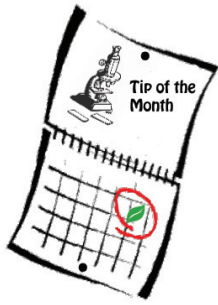
Federal Order for *Pseudomonas syringae* pv. *actinidiae*, Bacterial Canker of Kiwifruit

A Federal Order was issued on November 10, 2010, to prevent the introduction and dissemination of a bacterial canker of kiwifruit (*Pseudomonas syringae* pv. *actinidiae*) into the United States. APHIS will prohibit importations of *Actinidia* spp. plants for planting (including pollen but excluding fruit and seed) hosts of *P. syringae* pv. *actinidiae* (bacterial canker of kiwifruit) from all countries.

Recent scientific literature identifies *Pseudomonas syringae* pv. *actinidiae* as a destructive plant pathogen that is seriously affecting orchards of kiwi plants around the world. The disease is rapidly spreading though Asia and Europe. In Italy, it is estimated that the economic losses (including impact on trade) due to *P. syringae* pv. *actinidiae* have reached 2 million Euros.

Pseudomonas syringae pv. *actinidiae* is not known to occur in the United States. Plant pathogens, including viruses and viroids, are extremely difficult to detect during a port of entry inspection of the host plants, particularly in the absence of symptoms. The pathogen is not known to be seed or fruit transmitted.

[Click here](#) to read the official SPRO letter and Federal Order. 



Loop-mediated Isothermal Amplification (LAMP) as a Diagnostic Tool

Melodie Putnam,
Oregon State

University, Department of Botany and Plant Pathology

As a diagnostician I am always looking for assays that are rapid, accurate, easy to use, and inexpensive. Once I found out it is possible to have the specificity of PCR without the need for thermal cyclers, gels, or expensive and short-lived fluorescent probes using the method known as LAMP, I was definitely interested. Loop-mediated isothermal amplification (LAMP) takes place in a single microfuge tube and requires only a water bath or heat block. Results are evaluated visually without extensive manipulation of product. Since the method is the equivalent of a muscle car as far as speed (from a few copies of DNA to 10^9 , optimally, in less than an hour) and is highly specific, it seems to have the qualities that would be extremely useful in a diagnostic laboratory. We have developed an assay for detection of *Rhodococcus fascians* using this method (Serdani, *et al.*, 2009), are currently using LAMP for fire blight detection (Temple *et al.*, 2008), and have been very happy with both tests. The assay can be used on host tissue in addition to pure cultures, and it lives up to the claims for speed and selectivity. The following information is provided to pique your interest in developing your own assays.

LAMP was developed by Tsugunori Notomi of Eiken Chemical Company and colleagues at the University of Tokyo and Osaka University Medical School. Their seminal paper was published over ten years ago (Notomi *et al.*, 2000), and the method has since been adapted

for use with fungi (Tomlinson, *et al.*, 2010), nematodes (Kikuchi, *et al.*, 2009), and viruses (Fukuta *et al.*, 2003) in addition to phyto bacteria.

The process takes place in a microfuge tube containing a small amount of extracted sample DNA and four primers that are specific to six regions of your target DNA. A strand-displacing polymerase is used at 60-65°C (the specific temperature used depends on system optimization) and a series of looped structures result. The rather complex process of amplification and the structure formation is detailed at the Eiken website, which also includes an animation of the reaction (<http://loopamp.eiken.co.jp/e/lamp/index.html>). As a by-product of amplification, magnesium pyrophosphate accumulates in proportion to the amount of the specific product formed, and can be seen as turbidity in the reaction tube. If it is difficult to see this turbidity, a DNA binding dye may be used, which will integrate with the amplified product. This dye-DNA complex will fluoresce under UV light, which makes it easier to distinguish between positive and negative reactions.

For our LAMP assay for *Rhodococcus fascians*, we use either the FastSpin or Quiagen kits to extract DNA when starting with plant material, or if using a pure culture, simply boil the bacteria briefly to extract DNA. An aliquot is then added to the reaction mixture, the tubes are incubated at 60°C, and visualization is with PicoGreen (or the fluorescent dye of your choice). The entire process is

Diagnostic Updates



Positive LAMP reactions are shown in the two tubes on the left. The tube on the right is a negative reaction. The image was taken under UV illumination and fluorescence is due to addition of PicoGreen to all tubes.

completed in less than 3 hours. We have found the process to be fast, accurate, and very simple to use. However, the reaction products are extremely stable and can contaminate the laboratory in a persistent manner if you are not careful when opening the tubes to add the dye. This is best done in a room separate from where the DNA extraction occurs and where the reaction mixture is prepared.

There are web sites that can help you to design your own primers, one of which is at <http://primerexplorer.jp/elamp4.0.0/index.html>, and excellent technical information at the Eiken website: <http://loopamp.eiken.co.jp/e/lamp/index.html>.


References cited:

Fukuta S., Kato, S., Yoshida, K. Mizukami, Y., Ishida, A., Ueda, J., Kanbe, M., and Ishimoto, Y. 2003. Detection of tomato yellow leaf curl virus by loop-mediated isothermal amplification reaction. *Journal of Virological Methods* 112 (1-2):35-40. doi:10.1016/S0166-0934(03)00187-3

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* 28(12). doi: 10.1093/nar/28.12.e63.

Serdani, M., Curtic, M., and Putnam, M.L. 2009. Loop-mediated isothermal amplification (LAMP) for rapid detection of *Rhodococcus fascians* on ornamentals. *Phytopathology* 99:S117.


Temple, T.N., Stockwell, V.O. and Johnson, K.B. 2008. Development of a rapid detection method for *Erwinia amylovora* by loop-mediated isothermal amplification (LAMP) *Acta Hort.* (ISHS) 793:497-503.

Tomlinson, J.A., Dickinson, M.J., and Boonham, N. 2010. Detection of *Botrytis cinerea* by loop-mediated isothermal amplification. *Letters in Applied Microbiology* Dec;51(6):650-7. doi: 10.1111/j.1472-765X.2010.02949.x. 

National Plant Protection Laboratory Accreditation Program and 2011 *P. ramorum* Proficiency Test Panel

Patrick J. Shiel, USDA-APHIS-PPQ-CPHST

The National Plant Protection Laboratory Accreditation Program (NPPLAP) evaluates laboratories using molecular diagnostics on behalf of USDA-PPQ regulatory programs to ensure their capability for making accurate diagnostic determinations. In addition to ensuring lab capability within PPQ and other agencies in the USDA, NPPLAP engages the National Plant Diagnostic Network (NPDN) in this process to increase diagnostic capacity and proficiency. The goal of this program is to establish a state of readiness when needed by PPQ in emergency situations. NPPLAP also fosters the adoption of practices that promote continuous improvement and accreditation standards suitable for use by plant diagnostic labs and serves to develop functional Quality Assurance programs. NPPLAP began as the Provisional Approval process for the USDA *Phytophthora ramorum* Emergency Program and currently accredits laboratories to diagnose *Phytophthora ramorum* and the Huanglongbing (Citrus Greening) pathogen, with over 30 labs participating in one or both programs. The NPPLAP website is: www.aphis.usda.gov/plant_health/cphst/npplap.shtml. This website describes the program in detail and contains links to Inspection Checklists and other NPPLAP-related documents.

Visit the NPDN diagnostic website at www.npdn.org/diagnostics to read the 2011 *Phytophthora ramorum* panel information letter and to download a form for requesting panels. The documents can be found under the heading "Provisionally Approved Laboratories." 

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

Login and password required

Announcements ~ Membership information ~ Committee reports and meeting minutes ~ Documents and SOPs

DIAGNOSTICS COMMITTEE

Diagnostics Committee

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

The Diagnostics Committee held a conference call on November 4, 2010 and the following agenda items were discussed:

- IT-Diagnosticians Meeting/Ops Com overview
- 2011 NPDN Workshop(s) Update
- 2012 Future Workshop

- SOP Updates
- Surge Capacity Adobe Connect Training

Please refer to the website, www.npdn.org/diagnostics, for complete minutes of this meeting. The next conference call will be held on Thursday, December 9, 2010.

EXERCISE COMMITTEE

Exercise Committee

Sharon Dobesh, Program Area Manager/Committee Chair, Kansas State University, Department of Plant Pathology

The Exercise Committee conducted a conference call on November 16, 2010 and the following agenda items were discussed:

- Wrap up from August & September exercises
- Status of future APHIS-PPQ Exercises

- Status of State Exercises
- Other

The next conference call is scheduled for Tuesday, December 21, 2010.

EPIDEMIOLOGY COMMITTEE

Epidemiology Committee

Carla Thomas, Program Area Manager/Committee Chair, University of California at Davis, Department of Plant Pathology

The Epidemiology Committee held a conference call on November 3, 2010 and the following agenda items were discussed:

- Follow up on the NPDN IT-Diagnostician-Epidemiology meeting in Phoenix Oct.12-13
- Update on data analysis
- Update on data sharing

- Update on web tools and products
- Other items

The next Epidemiology conference call is scheduled for December 3, 2010. For complete minutes of this meeting visit the NPDN epidemiology page at www.npdn.org/epidemiology.

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 November 2, 2010. The subcommittee continues to work on reviewing the extensive NPDN pest lists. The agenda included:

- Discussion of change submissions

- Follow up on the IT/Diagnostician/Epidemiology meeting and enhancements to the software
- Changes to the Upload guidelines
- Discussion of the Abiotic Pest List

The next conference call will be held on December 15, 2010.

Training and Education Committee

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

The Training and Education Committee held a conference call on November 15, 2010 and the following agenda items were discussed on the call:

- Review committee for NPDN modules (Amanda)
- Strategies to broaden First Detector training
- First Detector award for the 2011 national meeting (Sharon)

- State accreditation for eLearning modules
- Poster for NDPN 2011 national meeting

The next conference call is scheduled for Monday, December 20, 2010.

Bioinformatics
Training
Postponed!



December 6-8, 2010 and December 8-10, 2010. The workshops have been postponed due to low attendance. They will most likely be offered in the early spring of 2011 along with a workshop for Citrus Greening. If you are interested in participating in any of these workshops please contact Karen Snover-Clift at kls13@cornell.edu.

NPDN-USDA APHIS 2010 Fall Training Sessions- Bioinformatics

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

The NPDN Diagnostics Program
Area Committee and members of
USDA-APHIS-PPQ-CHPST-National
Plant Germplasm and Biotechnology
Laboratory (NPGBL) had previously
scheduled a Bioinformatics workshop

PDIS New Features / Updates

Sharmila Dabade, Kansas State University, Department of Plant Pathology

The PDIS programming team would like to announce two new features.



1. Suspected Problem

When using the 'Suspected Problem' field on the lab form, PDIS will automatically suggest the previously used problems from that institution. This feature was added so that you can select from previously used suspected problems for consistency in wording of the problem or issue. Hopefully this will reduce the issue of having a number of entries for the same issue, such as "SOD Suspect", "SOD Survey", or "Sudden Oak Death Problem." If you have multiple data entry personnel, this new feature should benefit you. You still have the option of typing in a new problem in your own words.

Sample Number: 201003488 (Submitter: fname_Submitter; Host: Aaron's Beard)
NPDN No.: NPDN-9996-9192072; Checked-In By: User1_Diagnostician; Status: Preliminary; Submit Date: 8/10/2010

List of Tasks

- Submitter / Contacts
- Lab Form
- Images
- Transfer Sample
- Diagnosis & NPDN Upload
- Diagnostician/Consultant & Comments
- Reports
- Referrals
- Invoices
- Advanced NPDN Upload
- Replicate Current Sample
- Correspondence History
- View Sample Summary
- Close Current Sample Dashboard

Lab Form

Diagnosis Needed: Seed ID

Suspected Problem: **bl**

Host: black spots

Cultivar: black spots on the stem

Field/Reference ID: suspected problem

Sample Source: 3 matches found

Sample Category: Food/Furnishings-insectID

Sample Material Submitted:

Date Sample Collected:

Date Physical Sample Received:

Sample Collection Location

State: Kansas

County: Riley

Country: United States

Zip Code: 66502

Latitude: 45.1196

Create New Image

Enter detailed information for new Image for Sample Number 200902370



Image Information

Image Selection

Image1: C:\Documents and Sett Tag: Wheat

Image2: C:\Documents and Sett Tag: leaf rust

Image3: Tag:

Image4: Tag:

Diagnostician-Only Image :

Photographer : User1_Diagnostician *

Location

Zip : 66050

City : Manhattan

State : Kansas

County : Unknown

Country : United States



- **Image Type:** Please select only .png, .gif, .jpg, .jpeg, .bmp file for uploading image.
- **Location:** Enter location information from where the image has been taken. Entering Zip code will automatically fill County, State and Country.
- **Diagnostician-Only Image :** If checked, this image will be considered only for Diagnostician.



2. Image Tags

Diagnosticians requested a feature for searching images that are contained within samples. To address this request, a new identifier field called 'Tag' has been added when uploading sample images. One can add the keywords in the 'Tag' field, which will get associated with that image and allow you to search using the tag entered.

Update Image: 1721273

Edit information for the existing Image in Image List for Sample Number 200902370



Image Information

Image Description



Tag: wheat

Submission Date : 11/18/2010

Dimensions : 1230*2620

Submitted By : User1_Diagnostician
(demo1@acme.edu)

Institution : Acme Demonstration Institution

Diagnostician-Only Image :

Photographer : User1_Diagnostician *

Location

Zip : 66050

City : Manhattan

State : Kansas

County : Unknown

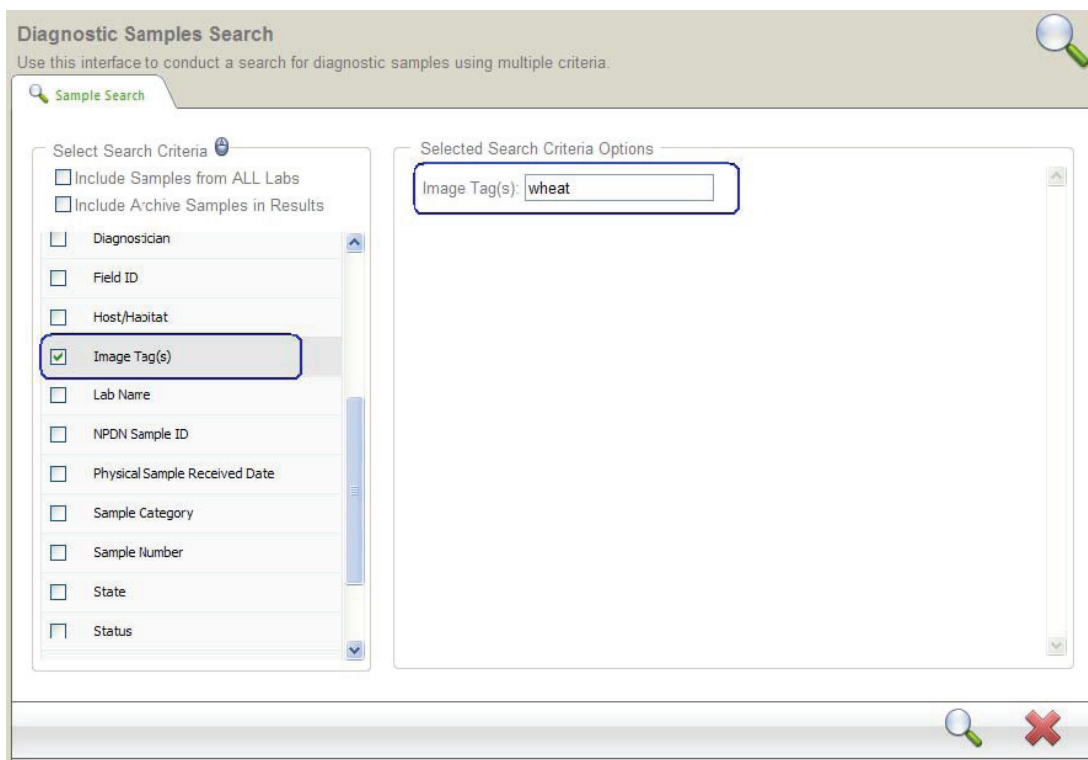
Country : United States



- **Image Type:** System supports only .png, .gif, .jpg, .jpeg, .bmp file for image.
- **Location:** Enter location information from where the image has been taken. Entering Zip code will automatically fill County, State and Country.
- **Diagnostician-Only Image :** If checked, this image will be considered only for Diagnostician.



Also, when you edit any existing image, you have the ability to add or edit a tag for that image.



Diagnosticians may use this identifier to search for sample images by selecting 'Image Tag(s)' from the search criteria. 🌿



Brown Marmorated Stink Bug Found in Minnesota

On November 22, 2010, Minnesota Department of Agriculture (MDA) announced that scientists found an unusual insect on some new equipment that had been delivered to their



Photo left: brown marmorated stink bug adult courtesy of David R. Lance, USDA-APHIS-PPQ, Bugwood.org.



Photo right: elongate hemlock scale courtesy of Eric R. Day, Virginia Polytechnic Institute and State University, Bugwood.org.

lab building. Staff from MDA's Plant Protection Division confirmed the insect was the first brown marmorated stink bug found in Minnesota.

[Click here](#) to read the announcement from MDA. 🌿

Regional News



Elongate Hemlock Scale Found in Maine Forest

For the first time, elongate hemlock scale was found in a natural

forest setting in Kittery Point, ME, which could create a threat to Maine's forest health. While it has been found in Kennebunkport and Kennebunk on landscape hemlock, it has never been found in the wild.

Elongate hemlock scale, an invasive insect from Asia, can also infest fir trees and although it has not yet been discovered on firs, its presence could eventually impact the state's commercial Christmas tree industry.

[Click here](#) to read the announcement at the Maine Forest Service website. 🌿



First U.S. Detection of Red Palm Weevil in California

On November 12, APHIS-PPQ announced that APHIS confirmed the detection of a dead red palm weevil (RPW), *Rhynchophorus ferrugineus*, on October 15, 2010. A local arborist reported the find in a tree stump in a residential area of Laguna Beach, Orange County, California. This is the first detection of RPW in the United States.

APHIS is working closely with the California Department of Food and Agriculture (CDFA) to determine the source and the extent of any potential infestation. On October 26, CDFA

inspectors found a single live adult RPW in a canary palm tree approximately 100 meters away from the original detection in Laguna Beach. Inspectors were alerted to the presence of several cocoons around the tree.



Exotic Fruit Fly Found in Florida

Florida Agriculture and Consumer Services Commissioner, Charles H. Bronson, announced on November 16, that a peach fruit fly, *Bactrocera zonata*, has been found in a trap in a guava tree in Miami-Dade County, Florida. The fly was found by a U.S. Department of Agriculture inspector during routine surveillance activities on November 10, 2010. This was the first find for this species in Florida and the the third exotic fruit fly find in Florida this year.

The peach fruit fly attacks many different fruits, vegetables and nuts, and is considered one of the most serious of the world's fruit fly pests due to its potential for economic harm.

[Click here](#) to read the announcement from the Florida Department of Agriculture and Consumer Services. Fact sheets and other information can be found at www.fl-dpi.com/enpp/ento/peach-fruit-fly.html. 🌿



Photo by CDFA Plant Pest Diagnostics Center - 2010

Photo courtesy of Plant Pest Diagnostics Center, CDFA.

Read [the full PPQ announcement here](#) or visit the California Department of Food and Agriculture website at www.cdafa.ca.gov/phpps/rpw/index.html and the Center for Invasive Species Research website at http://cisr.ucr.edu/red_palm_weevil.html for more information and excellent photos. 🌿

Associate Plant Pathologist (Diagnostician) with the California Department of Food and Agriculture, Plant Pest Diagnostics Center. [Click here](#) to view the full job announcement.

The Idaho State Department of Agriculture is currently recruiting for an **Agriculture Program Manager - Entomologist**. This position will be posted until December 15, 2010. [Click here](#) to review the job announcement.

The Nature Conservancy will be posting a job description for a **Forest Health Program Director**. The Director of the Forest Health Program helps coordinate and guide the Continental Dialogue on Non-native Insects and Diseases, oversees multiple outreach and policy campaigns focusing on prevention of introduction and spread of invasive pests and pathogens, and supervises several contractors and four staff.

The staff of the Forest Health program would like to invite anyone interested in this position to place themselves on a list of “interested parties.” The people on the interested parties list will directly receive an email notice when the job has officially opened on TNC’s hiring site (www.nature.org/careers/).

To add yourself to the list of interested parties:

Email Sarah Volkman, svolkman@tnc.org

In the subject line write “add to FH Program Director interested parties list”

Include in your email, your name & your preferred email address. Please do not send an attached cover letter or resume; these will not be read or used as part of the application process.

National Events

December 1-3, 2010
National CAPS Meeting
Kansas City, MO

December 12-15, 2010
ESA Annual Meeting
San Diego, CA

January 25-28, 2011
Nucleic Acid-Based Pathogen Workshop
Lexington, KY

November 6-9, 2011
NPDN National Meeting
San Francisco, CA

Regional Events

February 22-24, 2011
NEPDN Meeting
New Haven, CT

March 21-23, 2011
Soilborne Plant Pathogens and California
Nematology Workshop
Davis, CA

Job Opportunities

Upcoming Events

Rachel McCarthy, Editor
NEPDN
Cornell University