



# NPDN

National Plant Diagnostic Network



Advancing Diagnostics to Meet Plant Health Needs  
Fourth National Meeting • March 8-12, 2016 • Washington, DC

# NPDN Fourth National Meeting

## AGENDA AT A GLANCE

	Tuesday	Wednesday	Thursday	Friday	Saturday
7 am		Morning refreshments	Morning refreshments	Morning refreshments	Morning refreshments
8 am	Regional Meetings* <small>*see detailed agenda for more information</small>	Plenary Session	Increasing Capacity for Increased Food Security and Economic Stability, part I	Garden Walking Tour <small>Tour leaves hotel at 8:30am sharp!</small>	Workshops <small>Bus leaves hotel at 8am sharp! Workshops begin at 9am at the University of Maryland</small>
9 am					
10 am	Refreshment & Networking break	Refreshment & Networking break	Refreshment & Networking break		
11 am	Plenary Session	Plenary Session	Increasing Capacity for Increased Food Security and Economic Stability, part II	USDA Bus Tour <small>Bus leaves hotel at 8am sharp!</small>	
noon	Lunch on own	Lunch on own	Lunch on own		
1 pm	STAR-D Workshop	Advancing Diagnostics For Emerging Pathogens & Pests/Global Trade and Climate Change, part I	Novel Methods to Improve Prevention, Detection, & Diagnosis for Food Security and Trade, part I		
2 pm					
3 pm	Exhibit & Poster set up	Refreshment & Networking break	Refreshment & Networking break		
4 pm		Advancing Diagnostics For Emerging Pathogens & Pests/Global Trade and Climate Change, part II	Novel Methods to Improve Prevention, Detection, & Diagnosis for Food Security and Trade, part II		
5 pm					
6 pm	Welcome Social				
7 pm		Dinner on own	Awards Dinner		
8 pm	Dinner on own				
9 pm					

### REGISTRATION DESK HOURS

Tuesday 12noon – 5:30pm  
 Wednesday 7am – 5:30pm  
 Thursday 7am – 5:30pm



# WELCOME

## NPDN fourth national meeting

Welcome to the Fourth National Meeting of the National Plant Diagnostic Network (NPDN). Since our last national meeting in Berkeley, CA in November of 2011, the Network has formed new strategic connections and has continued to meet our goals and missions. Our education and outreach programs continue to educate a wide range of stakeholders, and we have developed and deployed new means of program delivery. With support from USDA-APHIS, we have been able to continue and expand on a series of excellent workshops for our diagnosticians. Our relationships and partnerships with State Departments of Agriculture and USDA-APHIS continue to grow. NPDN members add significantly to the growth of the Network and its impact through additional support from competitive grants and by partnering with researchers who are engaged in the development of diagnostics tools. This meeting will provide the opportunity for us to explore new and creative ways to address the challenges and opportunities that face the diagnostic community now and into the future.

Our national data repository is growing with over 1,000,000 diagnostic records. These records are providing a valuable resource for our diagnosticians and researchers as we monitor pests and diseases across the US. We have also reviewed our new governance structure and are working with a group of experts who serve as an external advisory council. During the last few years, the STAR-D accreditation program has been rolled out and several labs have gone through either full or gap-audits. It is great to see how far we have come toward meeting strategic goals to establish an internationally respected network of plant diagnostic laboratories. This overall record of accomplishment affirms the time, talents and commitment to excellence by all members of the NPDN.

A special thanks to the members of the various committees who have helped me plan and organize this meeting. These committees, with representatives from all five of the NPDN regions as well as our partners at USDA-NIFA, USDA-APHIS and State Departments of Agriculture, have spent many hours helping to plan this meeting. They have put together an exciting agenda with symposia, tours and workshops that we hope will be of interest to you.

Special thanks to the NPDN National Meeting committee chairs for their leadership—Carrie Harmon, Carla Thomas, Dave Clement, Rachel McCarthy and Sharon Dobesh. I also want to recognize Tamar Ditzian, Assistant Conference Coordinator from the University of Florida's IFAS Office of Conferences and Institutes, for her excellent help in managing the logistics of such a large meeting.

On behalf of the NPDN Executive Committee and all who helped organize this meeting, I hope that you will find this meeting to be enjoyable and productive for you. Please take time during the meeting to meet up with your colleagues from the US and around the world and make new connections as you learn more about the latest in diagnostics and the NPDN.

Once again, welcome to the meeting and thank you for your contributions to the development and success of the NPDN.

Best wishes and enjoy your meeting,

Ray Hammerschmidt

NPDN Executive Director, NCPDN Director, Michigan State University

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## Welcome to Washington, DC

The Organizers are excited to bring the 2016 NPDN meeting to the US capital. If you are traveling to the meeting site, we hope that you have a pleasant stay and make time to take in some of our national treasures.

## Metro Access

Washington, DC offers convenient transportation via the Metro Rail and bus system. The NPDN National Meeting at the DoubleTree by Hilton Hotel Washington DC - Crystal City, is just three blocks from the Pentagon City metro station.

See website [www.wmata.com](http://www.wmata.com)

## Hotel Shuttle

Although the hotel is within easy walking distance of the Metro, there is also a hotel shuttle available Monday through Friday, 6am – 11pm, and Saturday, Sunday and Holidays, 7am – 11pm. The shuttle departs every 30 minutes, on the hour and half hour. The Pentagon City Metro station pick-up and drop-off location is the bus stop at S Hayes Street and 12<sup>th</sup> Street S, Arlington, VA.

The 2016 program book was designed and edited by Rachel McCarthy & Dawn Dailey O'Brien, Cornell University. Cover design by Rachel McCarthy & Molly Towne.

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**hotel map**

## Tuesday, March 8

9:00am – 12noon	<b>Regional Meetings</b> GPDN regional meeting, LOCATION: Wilson *NEPDN regional meeting, LOCATION: Madison (*begins 8:30am) SPDN regional meeting, LOCATION: Van Buren WPDN regional meeting, LOCATION: Monroe
12noon – 1:00pm	Lunch on own
1:00pm – 5:00pm	<b>STAR-D Document Workshop</b> , LOCATION: Wilson
2:30pm – 5:30pm	Exhibit and poster setup in Crystal Ballroom
5:30pm – 7:00pm	<b>Welcome Social in Crystal Ballroom</b>
7:00pm	Dinner on own

## Wednesday, March 9

7:00am – 8:00am	Morning refreshments and networking in Crystal Ballroom
8:00am – 9:30am	<b>Plenary Session: Advancing Diagnostics to Meet Plant Health Needs: NPDN Partnerships, part I</b> LOCATION: Washington Ballroom
8:00am	<b>Welcome</b> <i>Carrie Harmon, NPDN Meeting Co-Chair</i>
8:15am	<b>Plant Health: A Priority for National and Global Food Security</b> <i>Sonny Ramaswamy, Director, National Institute of Food and Agriculture</i>
8:45am	<b>Safeguarding Plant Health While Protecting Global Trade</b> <i>Osama El Lissy, Deputy Administrator, APHIS PPQ</i>
9:30am – 10:30am	Refreshment and networking break in Crystal Ballroom
10:30am – 12noon	<b>Plenary Session: Advancing Diagnostics to Meet Plant Health Needs: NPDN Partnerships, part II</b> LOCATION: Washington Ballroom
10:30am	<b>NPDN's Positive Impacts on Diagnostics &amp; Extension Programming</b> <i>Ray Hammerschmidt, NPDN Executive Director &amp; NCPDN Director, Michigan State University, East Lansing, MI</i>
11:00am	<b>NPDN's Rapid &amp; Accurate Diagnostics in Support of Plant Production</b> <i>Joe Bischoff, Cornerstone Government Affairs, Washington, DC</i>

- 11:30am **NextGen NPDN: A National Network with Global Impact**  
*James Stack, GPDN Director, Kansas State University, Manhattan, KS*
- 12noon – 1:30pm Lunch on own
- 1:30pm – 3:00pm **Advancing Diagnostics For Emerging Pathogens and Pests Affected by Global Trade and Climate Change, part I**  
LOCATION: Washington Ballroom
- 1:30pm **The Lack of Walls: Grapevine Yellows, Palm Yellows and other Phytoplasmas, and the Helical Spiroplasmas**  
*Robert Davis, Research Leader, USDA ARS PSI MPPL, Beltsville, MD*
- Fuzzy Borers: Boring Insects that Vector Fungal Pathogens**
- 2:00pm **Thousand Cankers Disease**  
*Richard Bostock, WPDN Director, University of California, Davis, CA*
- 2:20pm **Tales of Two Hardwood Borers: Polyphagous Shot Hole Borer and Goldspotted Oak Borer**  
*Steven Seybold, USDA Forest Service, Davis, CA*
- 2:40pm **Holy Guacamole: Insights into the Emerging Laurel Wilt Pandemic**  
*Jason Smith, University of Florida, Gainesville, FL*
- 3:00pm – 3:45pm Refreshment and networking break in Crystal Ballroom
- 3:45pm – 5:30pm **Advancing Diagnostics For Emerging Pathogens and Pests Affected by Global Trade and Climate Change, part II**  
LOCATION: Washington Ballroom
- 3:45pm **Spreading the Heat: Insect Transmitted Pandemic-Associated Pathogens Concurrent with Changing Climate**  
*Judith K. Brown, University of Arizona, Tucson, AZ*
- 4:05pm **Seedy Business: Bioenergy as a New Source of Weeds**  
*Jacob Barney, Virginia Tech, Blacksburg, VA*
- Wiggle Room: Emerging Nematodes**
- 4:25pm **Newer Species of Root-Knot Nematodes**  
*Don Dickson, University of Florida, Gainesville, FL*
- 4:45pm **Importance of Nematodes on Turfgrasses and Landscape Plants**  
*Fred Warner, Michigan State University, East Lansing, MI*
- 5:05pm **A Multipurpose Pathogen: *Rathayibacter toxicus*: The Plant, Animal and Human Health Threat Nexus**  
*Ann Vidaver, University of Nebraska, Lincoln, NE*
- 5:30pm Dinner on own
- 5:45 – 7:15pm **Open Discussion on NPDN Diagnostic Confidence Levels & the National Data Repository** (Washington Ballroom)

## Thursday, March 10

- 7:00am – 8:00am Morning refreshments in Crystal Ballroom
- 8:30am – 10:00am **NPDN’s Role in Advancing Diagnostics: Increasing Capacity for Increased Food Security and Economic Stability, part I**  
LOCATION: Washington Ballroom
- 8:30am **KEYNOTE: Australia’s National Plant Biosecurity Diagnostic Strategy**  
*Steven Dibley, Program Manager for Training & Biosecurity Preparedness, Plant Health Australia*
- 9:00am **International Diagnostic Capacity Built on NPDN Capacity**  
*Tom Creswell, Purdue University, West Lafayette, IN & Carrie Harmon, University of Florida, Gainesville, FL*
- 9:30am **IPM Innovation Lab (CRSP) Diagnostics Training and Establishment in Developing Countries**  
*Sue Tolin, Virginia Tech, Blacksburg, VA*
- 10:00am – 10:45am Refreshment and networking break in Crystal Ballroom
- 10:45am – 12noon **NPDN’s Role in Advancing Diagnostics: Increasing Capacity for Increased Food Security and Economic Stability, part II**  
LOCATION: Washington Ballroom
- 10:45am **How to Get Your Lab Involved in Testing for Export or Import**  
*Weimin Ye, North Carolina Department of Agriculture & Consumer Services, Nematode Assay Lab, Raleigh, NC*
- The Role of NPDN in Diagnostic Capacity during Domestic Outbreaks**
- 11:10am **Combining Forces to Detect Invasive Forest Pests**  
*Deb McCullough, Michigan State University, East Lansing, MI*
- 11:30am ***Phytophthora ramorum***  
*Nancy Osterbauer, Oregon Department of Agriculture, Salem, OR & Norm Dart, Virginia Department of Agriculture, Richmond, VA*
- 12noon – 1:30pm Lunch on own
- 1:30pm – 3:00pm **Advancing Diagnostics using Novel Methods to Improve Prevention, Detection, and Diagnosis for Food Security and Trade, part I**  
LOCATION: Washington Ballroom
- 1:30pm **EDNA Methodology E-probe Diagnostic Nucleic Acid Analysis**  
*William Schneider, USDA ARS, Fort Detrick, MD*
- 1:50pm **Tracking Ug99 and Other Races of Wheat Stem Rust Using SNP Technologies**  
*Les Szabo, Cereal Disease Lab, USDA ARS, St. Paul, MN*

- 2:10pm **Multiplex for Tomato Diseases**  
*Chris Smart, Cornell University, Ithaca, NY*
- 2:30pm **Isothermal Analysis**  
*Paul F. Russel, Agdia, Inc., Elkhart, IN, Charla Hollingsworth, USDA APHIS CPHST, Raleigh, NC & Frank Martin, USDA ARS, Salinas, CA*
- 3:00pm – 3:45pm *Refreshment and networking break in Crystal Ballroom*  
**Poster and Exhibit removal (must be removed by 4pm!)**
- 3:45pm – 5:30pm **Advancing Diagnostics using Novel Methods to Improve Prevention, Detection, and Diagnosis for Food Security and Trade, part II**  
LOCATION: Washington Ballroom
- 3:45pm **The Impacts of Changes Due to Systematics on Fungal Identification**  
*Megan Romberg, National Mycologist, USDA APHIS, Beltsville, MD*
- 4:15pm **Morpho-Molecular Taxonomy Tools for Insects**  
*Ray Gill, California Department of Food and Agriculture, Sacramento, CA*
- 4:45pm **Scanning EM and Morphological-Molecular Techniques**  
*John Eisenback, Virginia Tech, Blacksburg, VA*
- 5:05pm **Diagnostics in the Information Age**  
*Mary Burrows, Montana State University, Bozeman, MT*
- 7:00pm – 9:00pm Awards Dinner in Crystal Ballroom

## Friday, March 11

- 7:30am – 8:30am Morning refreshments
- 8:00am – 5:00pm **Tours** — Meet in lower lobby, North Tower (Lincoln)
- USDA Bus Tour**  
*Tour Bus leaves hotel at 8:00am sharp!*
- Garden Walking Tour**  
*Tour leaves hotel at 8:30am sharp!*

## Saturday, March 12

- 7:30am – 8:30am Morning refreshments
- 8:00am – 5:00pm **Workshops** — Meet in lower lobby, North Tower (Lincoln)  
*Bus leaves hotel at 8:00am sharp! Workshops begin at UMD at 9:00am.*
- 5:00pm Conference concludes

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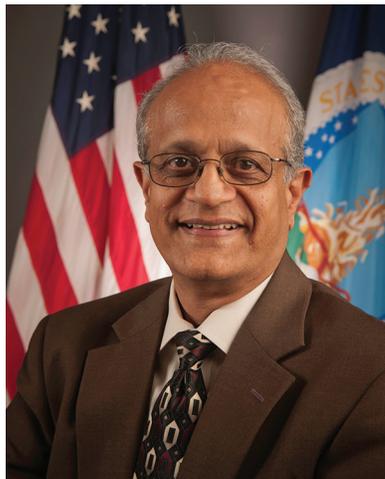
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Sonny Ramaswamy

**Dr. Sonny Ramaswamy** was appointed by President Barack Obama to serve as director of the National Institute of Food and Agriculture (NIFA). NIFA provides funding to catalyze transformative discoveries, education, and engagement to solve societal challenges.

Prior to starting at NIFA on May 7, 2012, Dr. Ramaswamy held a number of academic positions, including: dean of Oregon State’s College of Agricultural Sciences; director of Purdue’s Agricultural Research Programs; university distinguished professor and head of Kansas State’s Entomology Department; and professor of entomology at Mississippi State.

Sonny has been a successful scientist, educator, and administrator. He has received research grants from many federal agencies, including NIFA, NSF, NIH, EPA, and USAID, as well as from state agencies, commodity groups, and industry for his research in the area of integrative reproductive biology of insects. He has published over 150 journal articles, book chapters, and a book. He is an award-winning teacher, and has mentored a number of high school, undergraduate, graduate, and post-doctoral students.

He has received a number of awards and honors, including being named *Fellow of the American Association for the Advancement of Science* and *Fellow of the Entomological Society of America*.

Dr. Ramaswamy has had excellent success in capital campaigns and fund-raising to create endowments for faculty professorships, student scholarships and fellowships, including creation of the *Leadership Academy* at Oregon State University, support of research, extension, and outreach, infrastructure improvements, construction of new facilities for research, education, and outreach, including the Kansas State University *Insect Zoo*.

Dr. Ramaswamy received his Bachelor of Science degree in agriculture and Master of Science degree in entomology from the University of Agricultural Sciences, Bangalore, India. His doctorate is in entomology from Rutgers University. He is also a graduate of Harvard University’s Management Development Program. 🍃

**Osama El-Lissy** is the Deputy Administrator for the Animal and Plant Health Inspection Services’ Plant Protection and Quarantine (PPQ). In this position, he leads and directs a nationally dispersed staff that safeguards U.S. animal and plant resources from destructive pests and diseases.

For nearly two years before becoming PPQ’s Deputy Administrator, Osama was the Associate Deputy Administrator responsible for policy and financial management within PPQ. Before that, he directed PPQ’s emergency management, providing national coordination in the preparedness, response, and recovery from plant health emergencies in the United States. This included working with federal and state governments, industry stakeholders, and subject matter experts to develop and implement comprehensive plans designed to safeguard American agriculture against invasive pest outbreaks such as the recent citrus black spot in Florida, European grapevine moth in California, and potato cyst nematode in Idaho.

From 2000 to 2005, Osama served as the APHIS National Coordinator for cotton pest programs. In that role, he provided the coordination for national cotton pest programs in the United States and Mexico, including the boll weevil and pink bollworm eradication programs.

Osama’s previous experience includes more than twelve years in the private sector managing large scale pest control and eradication programs. As the director of the Texas boll weevil eradication program from 1994 through 2000, Osama led one of the largest pest eradication programs in the world, affecting approximately four million acres of cotton and providing services to several thousand cotton producers and landowners.

Osama earned a master’s degree in international business administration from Georgetown University, a master’s degree in public administration from American University, and a bachelor of science in agriculture production and entomology from Cairo University. 🍃



Osama El Lissy

**Ray Hammerschmidt** is professor of plant pathology in the Department of Plant, Soil and Microbial Sciences at Michigan State University (MSU). He previously served as chair of the department of Plant Pathology and most recently as the Interim Director of Michigan State University Extension. He is the Coordinator of Diagnostic Services at MSU and is the



Ray Hammerschmidt

founding and current director of the North Central Plant Diagnostic Network. He received his BS and MS degrees in biochemistry and plant pathology, respectively, from Purdue University, and his PhD in plant pathology from the University of Kentucky. Ray Hammerschmidt joined the faculty at MSU in 1980, where his research has focused on physiology and biochemistry of resistance and induced resistance in several plant systems. He has served as Major Professor for 11 PhD and 7 MS students and has mentored 9 post-doctoral research associates. He has served as Associate editor of *Phytopathology*, Senior Editor of APS Press and Senior Editor and Editor-in-Chief of *Physiological and Molecular Plant Pathology*. He was named a Fellow of the American Phytopathological Society in 2007, is co-recipient with many of his NPDN colleagues of the 2010 USDA-NIFA Innovative Programming Award, and also received the International IPM Award of Excellence. He teaches introductory plant pathology for undergraduates and a graduate course in plant-pathogen interactions. 🍃

**Dr. Joe Bischoff** joined the agriculture and natural resources team at Cornerstone Government Affairs in spring of 2015, where he consults for specialty and row crop grower groups, crop protection organizations, and academic institutions on federal agency activities and legislative issues. Previously, Joe was Regulatory & Legislative Affairs Director for AmericanHort, focused on the intersection of federal programs and science-based solutions to plant health and crop production challenges. He currently serves on the National Invasive Species Council's Invasive Species Advisory Committee (ISAC), was a member of the National Clean Plant Network's (NCPN) commodity committee for Fruit Trees and NCPN board member for Roses. Joe was on the research committee of the National Ornamentals Research Site at Dominican University of California (NORS-DUC) and has been a scientific reviewer and panel chair for research programs within the National Institute of Food and Agriculture (USDA-NIFA) and the Animal and Plant Health Inspection Service (USDA-APHIS). Before entering the private sector, Dr. Bischoff was with the USDA's Animal Plant Health Inspection Service (APHIS) where he was National Mycologist and Lead Scientist on the APHIS Intercepted Plant Pathogen Sequence Initiative (IPPSI). Following the completion of his doctoral degree from Rutgers University in 2004, Joe was the Fungal Taxonomist for GenBank, the National Institutes of Health's (NIH) genetic sequence database. He has authored over 30 peer-reviewed publications and was twice awarded the USDA-APHIS's Deputy Administrator's Safeguarding Award (2011, 2013), for his "exceptional work in safeguarding America's agricultural and natural resources." 🍃



Joe Bischoff

**Dr. Jim Stack** is Director of the Great Plains Diagnostic Network (GPDN), Professor of Plant Pathology, and Fellow of the Biosecurity Research Institute at Kansas State University. As Director of GPDN, Stack coordinates a nine-state project for the rapid detection and accurate diagnosis of high consequence plant pathogens and pests. He is principal investigator of plant biosecurity projects regarding emerging diseases and high consequence plant pathogens



Jim Stack

and is collaborating on international biosecurity projects in Europe, the Middle East, South America, Australia and New Zealand. Stack's research interests center on pathogen detection and surveillance, pathogen ecology and epidemiology for accidental and intentional (e.g., biological control, bioterrorism) introductions of plant pathogens into natural and agricultural plant systems. Research and scholarly interests span from preparedness and prevention to mitigation and recovery. Of particular interest is the intersection of plant health, public health, and food security. Prior to joining K-State, Stack was on the faculty at the University of Nebraska (1997–2003) and at Texas A&M University (1986–1989). Stack formerly worked for EcoScience Corporation as the Director of Applied Research, leading the discovery, development and commercialization of microbe-based disease management products that have been in commercial use for twenty years. 🍃



Stephen Dibley

**Dr. Stephen Dibley** joined Plant Health Australia (PHA), a not for profit company delivering biosecurity outcomes through collaborative working approaches with government agencies and peak agricultural and horticultural industry bodies, in 2008 from the University of Newcastle. He is currently the Program Manager for Training and Biosecurity Preparedness. Stephen manages the National Emergency Plant Pest Training Program, which delivers improved capability in PHA's member organisations to implement effective emergency responses to detections of new plant pests. The delivery of this program occurs through face-to-face training workshops, simulation exercises and e-learning platforms. Stephen also contributes to emergency response preparedness in his role as the Deputy Chair of the Subcommittee on Plant Health Diagnostics (SPHD), the peak national group for plant health diagnostics in Australia. In this role, Stephen contributed to the development of the National Plant Biosecurity Diagnostic Strategy and the implementation of the National Plant Biosecurity Diagnostic Network (NPBDN). The NPBDN connects the plant health diagnosticians across the country and delivers professional development opportunities to its members.

Stephen's academic training and research background covers a career in plant molecular biology, through a Bachelor of Science (Biotechnology) with Honours and the completion of a PhD focussed on the role of sugar transporters in developing tomato fruit. His post-doctoral research at the University of Newcastle covered sugar transport and cell wall biosynthesis in bean seeds. Stephen has maintained a passion for sharing knowledge throughout his career, starting in the university sector and continuing in his current role. 🌿

## AUSTRALIA'S NATIONAL PLANT BIOSECURITY DIAGNOSTIC STRATEGY

**Stephen Dibley<sup>1</sup>, Deborah Hailstones<sup>2</sup>, Mike Hodda<sup>3</sup>, Douglas Kerruish<sup>4</sup>, Jo Luck<sup>5</sup> and Barbara Hall<sup>6</sup>**

<sup>1</sup>Plant Health Australia, Canberra, ACT, Australia; <sup>2</sup>New South Wales Department of Primary Industries, Menangle, NSW, Australia; <sup>3</sup>CSIRO, Canberra, ACT, Australia; <sup>4</sup>Australian Government Department of Agriculture and Water Resources, Canberra, ACT, Australia; <sup>5</sup>Plant Biosecurity Cooperative Research Centre, Melbourne, VIC, Australia; <sup>6</sup>South Australian Research and Development Institute, Adelaide, SA, Australia

Australia's National Plant Biosecurity Strategy was finalized in 2010 to present a vision for the plant biosecurity system through to 2020. Extensive stakeholder consultation with governments and plant production industries over three years was essential to its development. As it represents a shared vision, the strategy has received the support to strengthen the biosecurity system considerably.

A key element underpinning the overall strategy is the National Plant Biosecurity Diagnostic Strategy (NPBDS). The NPBDS explicitly publishes the agreed recommendations and actions necessary to ensure Australia has the people, infrastructure, diagnostic standards and tools to improve the delivery of plant biosecurity diagnostic services. The NPBDS has strengthened biosecurity by promoting and implementing systems and practices that have increased confidence in diagnostic outcomes, regularly tested through a proficiency testing program. The NPBDS has also supported the development and revision of National Diagnostic Protocols for 34 high priority exotic pests, which have been endorsed for use in emergency responses to provide definitive pest identification.

The National Plant Biosecurity Diagnostic Network (NPBDN), officially launched in 2011, has been vital to the implementation of the NPBDS. The NPBDN connects plant health diagnosticians from more than 70 locations and institutions across Australia to deliver and improve services. It's a flexible, self-organizing network, with formal oversight and regulation. The overseers of the NPBDN also organize and deliver professional development in areas of strategic need, support short-term residential laboratory exchanges, and bring many of the diagnosticians together for an annual workshop themed around common general diagnostic techniques or issues.

Contact Information: Stephen Dibley, Plant Health Australia, Level 1, 1 Phipps Close, Deakin, ACT, Australia 2600, Phone: +61 2 6215 7709, Email: [sdibley@phau.com.au](mailto:sdibley@phau.com.au)

NO.	FIRST NAME	LAST NAME	ORGANIZATION	POSTER TITLE
<b>Advancing Diagnostics to Meet Plant Health Needs: NPDN Partnerships and Projects</b>				
35	Gloria	Abad	USDA APHIS PPQ S&T CPHST	ONLINE MORPHOLOGICAL AND MOLECULAR TOOLS FOR <i>PHYTOPHTHORA</i> : MEETING THE NEEDS FOR AN INNOVATIVE RESOURCE TO AID IN THE ACCURATE IDENTIFICATION OF SPECIES IN THE GENUS
39	Mohammad	Arif	Kansas State University	INTERNAL CONTROL: ESSENTIAL OR OPTIONAL FOR ACCURATE AND RELIABLE DIAGNOSTICS
31	Elizabeth	Bush	Virginia Tech	A PLANNED DEVIATION OF A CPHST-VALIDATED PROTOCOL ALLOWS FOR USE OF A HIGH-THROUGHPUT REAL-TIME MULTICYCLER FOR DETECTION OF <i>PHYTOPHTHORA RAMORUM</i>
38	Grethel	Busot	Kansas State University	NUCLEIC ACID-BASED DIAGNOSTICS: DOES THE TARGET SEQUENCE MATTER?
32	Emmanuel	Byamukama	South Dakota State University	PARTNERING WITH THE STATE SOYBEAN COMMODITY BOARD TO PROMOTE DIAGNOSIS AND MANAGEMENT OF SCN IN SOUTH DAKOTA
41	David	Clement	University of Maryland	MARYLAND INVASIVE TRAINING AND OUTREACH PROGRAMS
48	Tom	Creswell	Purdue University	BEYOND 'NATIONAL': THE NPDN AT WORK ABROAD
36	Shefali	Dobhal	Kansas State University	ESSENTIALS OF MOLECULAR DIAGNOSTIC PROTOCOL VALIDATION TO DEVELOP RELIABLE AND ACCURATE DIAGNOSTICS
44	Snezana	Haymes	USDA-APHIS-PPQ-CPHST	MOLECULAR DIAGNOSTICS FOR <i>MONILINIA</i> SPECIES OF REGULATORY CONCERN
40	Ann	Hazelrigg	University of Vermont	PROMOTING IPM THROUGH A PARTNERSHIP BETWEEN THE UVM PLANT DIAGNOSTIC CLINIC AND THE VERMONT EXTENSION MASTER GARDENER PROGRAM

NO.	FIRST NAME	LAST NAME	ORGANIZATION	POSTER TITLE
43	Richard	Hoenisch	Western Plant Diagnostic Network	WPDN MALACOLOGY WORKSHOPS & TRAINING AND EDUCATION TRAINING AND CONNECTING IN THE WEST
42	Zhonghua	Liu	PathSensors, Inc.	DEVELOPING CANARY ASSAYS FOR PLANT PATHOGEN DETECTION
30	Vessela	Mavrodieva	USDA APHIS PPQ	THE NPPLAP PROFICIENCY TESTING PROGRAM: TEN YEARS OF EXPANDING LABORATORY CAPACITY AND PROMOTING QUALITY FOR REGULATED PLANT PATHOGENS TESTING
33	Rachel	McCarthy	Cornell University	SENTINEL PLANT NETWORK: INCREASING EARLY DETECTIONS THROUGH TRAINING AND OUTREACH
45	Cynthia	Music	CERIS/Purdue University	NPDN DATABASE COMMITTEE; ASSURING QUALITY AND STANDARDS IN NPDN DIAGNOSTICS DATA
49	Jennifer	Olson	Oklahoma State University	PICTORIAL GUIDE TO ROSE ROSETTE DISEASE SYMPTOMS
14	Melodie	Putnam	Oregon State University	GALL-ID: WEB-BASED TOOLS FOR THE RAPID IDENTIFICATION AND CHARACTERIZATION OF GALL-CAUSING PHYTOPATHOGENIC BACTERIA
37	Lina	Rodriguez-Salamanca	Iowa State University	EXPANDING THE IMPACT OF NPDN: DIAGNOSTIC TRAINING IN COLOMBIA
34	Barbara	Shew	North Carolina State University	PLANTS, PESTS AND PATHOGENS WEBINARS AT NC STATE UNIVERSITY
10	Karen	Snover-Clift	Cornell University	BELTSVILLE WORKSHOPS; AN NPDN AND USDA-APHIS-PPQ-S&T COLLABORATIVE EFFORT TO STRENGTHEN NATIONAL DIAGNOSTIC READINESS
11	Karen	Snover-Clift	Cornell University	STAR-D; THE CREATION AND IMPLEMENTATION OF NPDN'S LABORATORY ACCREDITATION PROGRAM FOR PLANT DIAGNOSTIC LABORATORIES
47	James	Stack	Kansas State University	DEVELOPING DURABLE DNA-BASED DIAGNOSTICS: HOW WELL DO YOU KNOW YOUR SOP?
46	Shouhua	Wang	Nevada Department of Agriculture	BUILDING AND EVOLVING PLANT DIAGNOSTICS TO SERVE NEVADA'S AGRICULTURE AND URBAN PLANT HEALTH

NO.	FIRST NAME	LAST NAME	ORGANIZATION	POSTER TITLE
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## EMERGING PESTS



The invasive goldspotted oak borer, *Agrilus auroguttatus*, is threatening the health and survival of oak trees in San Diego County, California.

Goldspotted oak borer adult collected in San Diego County 2010. photo © Mike Lewis, Center for Invasive Species Research, Bugwood.org

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**ONLINE MORPHOLOGICAL AND MOLECULAR TOOLS FOR PHYTOPHTHORA: MEETING THE NEEDS FOR AN INNOVATIVE RESOURCE TO AID IN THE ACCURATE IDENTIFICATION OF SPECIES IN THE GENUS****Z. Gloria Abad and John C. Bienapfl**

USDA-APHIS-PPQ-Center for Plant Health, Science &amp; Technology-Beltsville Laboratory, Beltsville, MD

The genus *Phytophthora* currently contains 152 species, many of which cause significant economic impact to crops, ornamentals, and forests. Many species are globally recognized as being of regulatory concern, including 29 hierarchically ranked by the CPHST Plant Epidemiology and Risk Analysis Laboratory (PERAL) in 2009. Since the CPHST-PERAL list was released, 58 new species have been described, some of which may also be of concern. Considerable progress has been made in the identification, phylogenies, and diagnostics of *Phytophthora* species in the past decade, but this work is still challenging due to omissions of the ex-type specimens in taxonomic publications and numerous misidentifications based on DNA sequences submitted to GenBank and other databases. Correct identification for members of some species complexes (i.e. *P. capsici*, *P. citricola*, *P. crytozea/P.drechleri*, *P. megasperma*, *P. parsiana*, etc.) can be especially difficult. The CPHST Beltsville Laboratory is pioneering work with international collaborators for the implementation of the “Online Identification Tools for *Phytophthora*: Lucid Key, Tabular Key, and Sequencing Analysis” (based on the cultures of the ex-types and DNA obtained from these cultures) to offer correct species identification. Our ultimate goal is to provide a solid basis for the implementation of robust identification and diagnostic systems for *Phytophthora* species of concern. A workshop to beta-test the Online Identification Tools for *Phytophthora* will be presented during the 2016 NPDN national meeting and the official release of the resource will coincide with a workshop on identification of *Phytophthora* species presented at the 2016 annual American Phytopathological Society meeting.

Contact Information: Z. Gloria Abad, USDA-APHIS-PPQ-Center for Plant Health, Science & Technology-Beltsville Laboratory, Beltsville, MD, Phones: Office: 301-313-9340, Cell: 301-379-8918, E-mail: Gloria.abad@aphis.usda.gov

**SEEDBORNE FUNGI ISOLATED FROM PULSE CROP SEEDS IN MONTANA****Bright Agindotan and Mary Burrows**

Dept. Plant Pathology and Plant Sciences, Montana State University, Bozeman, Montana

Pulse crops (chickpea, lentil, and field pea) are grown globally for their health and nutritional values. Montana is the leading producer and exporter of field peas and lentils in the United States. In 2014, Montana produced 52.9% (1,761,000 cwt), 52.3% (3,367,000 cwt), 16.9% (475,000 cwt) of U.S. field peas, lentils, and chickpeas, respectively. Area planted with field pea increased from 540,000 acres in 2013 to 543,000 acres in 2014. As the acreage of any crop increases, so is the risk of infection by pests. Growers in Montana are aware of the fungal disease *Ascochyta/Mycosphaerella* blight and have been testing their seeds exclusively for causal agents of this disease for years. This has benefited growers by eliminating heavily infected seed lots from the planted acres and reducing disease inoculum. However, there is a fear that other seedborne pathogens have been allowed to flourish. To determine the prevalence of other seedborne pathogens, in 2015 growing season, the Regional Pulse Crop Diagnostic Laboratory fungal-scanned all seed samples sent to it so far (60 field pea, 27 lentil, and 12 chickpea). The most prevalent pathogens isolated from the pulse crops were *Alternaria* spp. (86.7%–88.9%), *Cladosporium* spp. (77.8%–86.7%), *Ascochyta/Mycosphaerella* spp. (22.2%–76.7%), *Fusarium* spp. (21.7%–22.2%), *Penicillium* spp. (21.7%–22.2%), and *Stemphylium* spp. (20.0%–29.6%). Growers can manage these fungal diseases if they know the health status of their seedlots. We recommend growers request for fungal scan of their seedlots from seed testing laboratories.

Contact Information: Bright Agindotan, Montana State University, 1911 W. Lincoln St., Bozeman, MT 59718-3145, Phone: 406-994-5162, Email: bright.agindotan@montana.edu

## **NPDN FIRST DETECTORS: NEW AND UPDATED RESOURCES FOR ENHANCED PEST AND PATHOGEN DETECTION**

**Joan Allen**<sup>1</sup>, Rachel McCarthy<sup>2</sup>, Dick Hoenisch<sup>3</sup>, Sharon Dobesh<sup>4</sup>, David Clement<sup>5</sup>, G. Keith Douce<sup>6</sup>, Mike Hill<sup>7</sup>, Joseph LaForest<sup>6</sup>, Brett Arenz<sup>8</sup>, Jennifer Olson<sup>9</sup>, Lina Rodriguez-Salamanca<sup>10</sup>, Ron French<sup>11</sup>, Marty Draper<sup>12</sup>, Carrie Harmon<sup>13</sup>

<sup>1</sup>University of Connecticut, Storrs, CT; <sup>2</sup>Cornell University, Ithaca, NY; <sup>3</sup>University of California Davis, Davis, CA; <sup>4</sup>Kansas State University, Manhattan, KS; <sup>5</sup>University of Maryland, College Park, MD; <sup>6</sup>University of Georgia, Tifton, GA; <sup>7</sup>Purdue University, West Lafayette, IN; <sup>8</sup>University of Minnesota, Duluth, MN; <sup>9</sup>Oklahoma State University, Stillwater, OK; <sup>10</sup>Iowa State University, Ames, IA; <sup>11</sup>Texas A&M AgriLife Extension Service, Amarillo, TX; <sup>12</sup>USDA-NIFA, Washington D.C.; <sup>13</sup>University of Florida, Gainesville, FL; <sup>14</sup>Michigan State University, East Lansing, MI

The National Plant Diagnostic Network (NPDN) Training & Education Committee creates and updates diagnostic and detection resources for First Detectors, educators and others to increase the likelihood of early detection and identification of unknown or high-consequence plant pests and pathogens. These pests and pathogens cost the United States billions of dollars annually and early detection reduces the impact of pesticides on our food supply, lessens the destruction of our natural environments and reduces the economic impact from plant losses. Resources available include both online and scripted PowerPoint presentations for First Detector training, workshop registration tools for First Detector educators, and images and information on pests and pathogens of concern in the form of fact sheets, pest alerts, posters, ID guides, YouTube videos and website links. Diagnosticians and industry professionals will be interested in the advanced identification training videos and the suite of educational materials on the Asian longhorned beetle. A new series of materials, “Sharpening Your Observation Skills” is now available that highlights key identification features between several significant pests with native look-alike species. Some of these resources have been translated into Spanish and many are compatible with smartphones and tablets for convenient use in the field. Training and education materials are available at [www.firstdetector.org](http://www.firstdetector.org).

Contact Information: Joan Allen, University of Connecticut, 1380 Storrs Rd., Unit 4115, Storrs, CT 06269-4115, Phone: 860-486-6740, Email: [joan.allen@uconn.edu](mailto:joan.allen@uconn.edu)

## **UCONN DIAGNOSTIC LAB: THE NEW, UNUSUAL OR PREVIOUSLY UNENCOUNTERED**

**Joan Allen**<sup>1</sup>, Wade Elmer<sup>2</sup>, Jude Boucher<sup>1</sup>

<sup>1</sup>University of Connecticut, Storrs, CT; <sup>2</sup>Connecticut Agricultural Experiment Station, New Haven, CT

In the Plant Diagnostic Lab, every year or every season brings a unique variety of problems. Year after year, a handful of pest and disease problems come through the door that haven't been seen in the lab before. It may be something relatively common or it could be something that really is rare or new in Connecticut. Featured samples include Fusarium wilt of fragrant sumac (*Fusarium oxysporum*), spinach crown mite (*Rhizoglyphus* sp.), bacterial canker of pepper (*Clavibacter michiganensis* pv. *michiganensis*), blister beetles on Brassicas (*Meloe campanicollis*), Phoma dieback on boxwood, dagger nematode (*Xiphinema* sp.) on raspberry and others.

A sample of fragrant sumac (*Rhus aromatica*) was received from a client in Massachusetts in fall 2014. Symptoms included leaf scorch, browning of the vascular tissue and gummosis from branches. Sporodochia with macroconidia were present in lenticels on the bark of symptomatic branches and the fungus was identified as *Fusarium oxysporum* by Wade Elmer. Koch's postulates have been completed and molecular analysis is pending. These symptoms and signs were similar to those described for Mimosa wilt caused by *F. oxysporum* f.sp. *perniciosum*.

Spinach crown mite and bacterial canker of pepper were both diagnosed for the first time in Connecticut in 2015. Spinach crown mite is a bulb mite and can survive on either organic matter in the soil or on living plant material. Bacterial canker of pepper has been reported recently from several states and was confirmed from a farm in Connecticut this year. These problems and others will be featured in this presentation.

Contact Information: Joan Allen, University of Connecticut, 1380 Storrs Rd., Unit 4115, Storrs, CT 06269-4115, Phone: 860-486-6740, Email: [joan.allen@uconn.edu](mailto:joan.allen@uconn.edu)

## BEYOND PHYTOPHTHORA RAMORUM: IDENTIFYING OTHER PHYTOPHTHORA SPECIES, SEARCHING FOR P. KERNOVIAE AND EVALUATING SPECIES LEVEL TESTING METHODS

Tricia Allen<sup>1</sup>, Karen Snover-Clift<sup>1</sup>, Margery Daughtrey<sup>2</sup>, Sandra Jensen<sup>1</sup>

<sup>1</sup>Cornell University, Section of Plant Pathology and Plant-Microbe Biology, Plant Disease Diagnostic Clinic (PDDC), Ithaca, NY; <sup>2</sup>Cornell University, Long Island Horticultural Research and Extension Center, Riverhead, NY

Beginning in 2004, the Cornell University's Plant Disease Diagnostic Clinic (PDDC) has provided *Phytophthora ramorum* identification testing for numerous state and national surveys, trace forward/back events and Farm Bill projects. Since 2004, 3,695 suspect *P. ramorum* samples have been processed. Working with so many samples has triggered questions in regards to the process and our findings. Currently only the ELISA procedure is accepted by regulatory agencies for preliminary testing to the *Phytophthora* genus level. However, the ImmunoStrip® is an ideal diagnostic tool for small sample sets, therefore, a comparison of results may allow this alternative procedure. Furthermore, ELISA testing indicated that many of the samples processed contained a *Phytophthora* species but no species identification testing was done due to the additional cost of labor and supplies needed. Another significantly harmful *Phytophthora* species, *P. kernoviae*, has not yet been identified in the United States; monitoring for it is not common practice. Testing plants for *P. kernoviae* is important because this pathogen is reportedly much more damaging; it was found in Europe during their *P. ramorum* surveys. A Specialty Crop Block Grant allowed us to accomplish three objectives, using our 2014 samples: 1) sequence *Phytophthora* positive samples, 2) test samples using qPCR ITS1 and ITS2 protocols for *P. kernoviae*, and 3) compare the ELISA *Phytophthora* species procedure with the ImmunoStrip® test. This project allowed us to name 14 different *Phytophthora* species detected from 205 isolates, determine no *P. kernoviae* was present in these samples and show a few differences between results of ELISA versus ImmunoStrip®.

Contact Information: Tricia R. Allen, Cornell University, 334 Plant Science Bldg. Ithaca, NY 14853, Phone: 607-255-7850, Email: tra42@cornell.edu

## INTERNAL CONTROL: ESSENTIAL OR OPTIONAL FOR ACCURATE AND RELIABLE DIAGNOSTICS

Mohammad Arif, Grethel Y. Busot, Shefali Dobhal, and James P. Stack

Department of Plant Pathology, Kansas State University, Manhattan, KS

Plant diseases are an essential part of nature keeping hundreds of thousands of plants and animals in balance. Plant disease management depends on an accurate identification of the causal agent(s). Identification of many pathogens based solely on morphological characteristics (e.g., bacteria) carries a high probability of inaccuracy and may require expertise in host and/or pathogen taxonomy. Nucleic acid-based diagnostic tests, for example PCR, provide a high level high of accuracy and reliability and have become routine in plant diagnostic labs. However, reliability of PCR-based diagnostics depends on the accuracy of standard operating protocols (SOPs). PCR can generate false negatives or false positives due to a variety of error mechanisms including, inefficient DNA extraction, presence of PCR inhibitors in the reaction, poorly designed primers sequences, and reagent failure. The use of internal controls (IC) in PCR can facilitate interpretation and increase the confidence in the results obtained. Ideally, two internal controls are required in each reaction: one to verify successful DNA extraction and the other to verify that the PCR reaction was free of inhibitors. Different approaches for ICs have been developed and a variety of ICs are used by diagnosticians and scientists in diverse assays. However, the inclusion of traditional ICs in PCR in a diagnostic laboratory setting can be expensive and time consuming due to the large array of potential target pathogens and host plants for which internal controls must be maintained. Multi-target ICs based on multiple pathogens and plants are inexpensive and easy to maintain in the lab.

Contact Information: Mohammad Arif, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, Phone: 785-532-1380, Email: arif@ksu.edu

**THE NATIONAL PLANT DIAGNOSTIC NETWORK DIAGNOSTICS PROGRAM AREA COMMITTEE**

**Clarissa Balbalian**<sup>1</sup>, Frank Hale<sup>2</sup>, Gail Ruhl<sup>3</sup>, Sladana Bec<sup>4</sup>, Tamla Blunt<sup>5</sup>, Jan Byrne<sup>6</sup>, Kassie Conner<sup>7</sup>, Jason French<sup>8</sup>, Carla Garzon<sup>9</sup>, Fanny Iriarte<sup>10</sup>, Ansuya Jogi<sup>11</sup>, Janet Lamborn<sup>12</sup>, Laurene Levy<sup>13</sup>, Sara May<sup>14</sup>, Judy O'Mara<sup>15</sup>, Melodie Putnam<sup>16</sup>, Karen Rane<sup>17</sup>, Lina Rodriguez-Salamanca<sup>18</sup>, Pat Shiel<sup>19</sup>, Karen Snover-Clift<sup>20</sup>, Raj Singh<sup>21</sup>, and Carla Thomas<sup>22</sup>

<sup>1</sup>Mississippi State University, Mississippi State, MS; <sup>2</sup>University of Tennessee, Nashville, TN; <sup>3</sup>Purdue University, West Lafayette, IN; <sup>4</sup>University of Florida, Gainesville, FL; <sup>5</sup>Colorado State University, Fort Collins, CO; <sup>6</sup>Michigan State University, East Lansing, MI; <sup>7</sup>Auburn University, Auburn, AL; <sup>8</sup>New Mexico State University, Las Cruces, NM; <sup>9</sup>Oklahoma State University, Stillwater, OK; <sup>10</sup>University of Florida, Quincy, FL; <sup>11</sup>University of Georgia, Athens, GA; <sup>12</sup>Agida, Inc., Elkhart, IN; <sup>13</sup>USDA, APHIS, PPQ, CPHST, Riverdale, MD; <sup>14</sup>Pennsylvania State University, University Park, PA; <sup>15</sup>Kansas State University, Manhattan, KS; <sup>16</sup>Oregon State University, Corvallis, OR; <sup>17</sup>University of Maryland, College Park, MD; <sup>18</sup>Iowa State University, Ames, IA; <sup>19</sup>USDA, APHIS, PPQ, CPHST, Raleigh, NC; <sup>20</sup>Cornell University, Ithaca, NY; <sup>21</sup>Louisiana State University, Baton Rouge, LA; <sup>22</sup>University of California, Davis, CA

Since its establishment in 2003, the Diagnostics Program Area Committee has focused on a number of different initiatives, including communication between diagnosticians, lab acquisition of USDA permits, creating standard operating procedures (SOPs) for diagnosis of significant regulated pathogens, coordinating select agent workshops, coordinating laboratory surge capacity, developing a basic techniques workshop for diagnosticians and creating a working group to assist in the creation of the STAR-D laboratory accreditation program. The committee, in collaboration with our USDA and University partners, also has developed a series of workshops conducted at the USDA laboratory in Beltsville, MD that train NPDN diagnosticians to use morphological and molecular testing for highly significant pathogens. The primary focus of the diagnostics program area committee is to provide diagnosticians with opportunities and information necessary to achieve the NPDN mission of providing timely, accurate diagnostics. Our committee members strive to keep NPDN diagnosticians and collaborators informed and prepared for identifying new pathogens, processing significant events and recognizing behavioral modifications in endemic pathogens and pests.

Contact Information: Clarissa Balbalian, Extension Plant Pathology Lab, 190 Bost North, Box 9612, Mississippi State, MS 39762-9612, Phone: 662-325-2146, Email: c.balbalian@msstate.edu

**SAMPLE SUBMISSION GUIDELINES AND PROCESSES FOR TIMELY AND ACCURATE CONFIRMATORY DIAGNOSTICS FROM THE CPHST BELTSVILLE LAB**

**Ashlee Barth**, Deric D. Picton and Mark K. Nakhla

USDA APHIS PPQ S&T CPHST Beltsville Laboratory, Beltsville, MD

Accurate and timely submission of diagnostic samples requiring US Federal confirmation is essential to ensure that quality results are generated by the CPHST Beltsville Laboratory, the only ISO 17025 Accredited federal plant pathogen diagnostic lab within USDA-APHIS-PPQ. Proper documentation of the sample type and suspected pathogen is required for the Beltsville Lab to determine what resources need to be allocated to providing timely confirmations. Proper documentation also allows the Beltsville Lab to consult with PPQ officials to determine if a particular sample requires molecular based federal confirmation. The use of the list of pathogens of concern to the United States is very useful to determine whether a particular pest sample requires submission and Federal confirmation. This list is generated by APHIS-PPQ program and they can provide guidance to NPDN labs upon request.

Contact Information: Ashlee Barth, USDA APHIS PPQ CPHST-Beltsville, Bldg. 580 BARC-East Powder Mill Road, Beltsville, MD 20705-2350, Phone: 301-313-9227, Email: Ashlee.k.barth@aphis.usda.gov

**EFFECTIVE MANAGEMENT OF PESTS AND HARMFUL ALIEN SPECIES - INTEGRATED SOLUTIONS (EMPHASIS)**

**Neil Boonham**<sup>1</sup>, Michael Andreou<sup>2</sup>, Tom Woods<sup>3</sup>, Jane Thomas<sup>3</sup> and Davide Spadero<sup>4</sup>

<sup>1</sup>Fera, Sand Hutton, UK; <sup>2</sup>OptiSense Limited, Horsham, UK; <sup>3</sup>NIAB, Cambridge UK; <sup>4</sup>Centro Agrinnova, Largo Braccini, Italy

EMPHASIS is an EU funded interdisciplinary research project with the key objectives of developing novel approaches to the early detection, integrated control and socio-economic assessment of the threats to European agriculture

and the environment from indigenous and alien plant pests and pathogens. Within work-package 2 we are focusing on developing targeted and untargeted methods for monitoring and surveillance respectively to enable the early detection of incursions of a range of target pests and pathogens. In terms of surveillance the project will investigate the use of an EU wide network of sentinel plots for detection and isolation of risky airborne fungal pathogens. Development will also be done linking together existing trap networks for insects and airborne inoculum (e.g. pollen and air-quality traps) linked to meta-barcoding approaches to identify the trap contents in an untargeted fashion. The aim is to provide broad surveillance approaches for a number of customers (e.g. NPPOs, farmers, agronomists) using established shared cost networks with the aim of providing low cost data for each sector. In addition work will be performed developing and validating (to international EPPO standards) a large range of 'in-field' tests for detection and identification of pathogens and pests based on LAMP chemistry deployed on the Genie II & III platforms. Development of the platform will also be carried out improving the user experience with automated results calling and on screen 'walk through' protocol videos to help inexperienced users (e.g. agronomists or inspectors) to more rapidly become familiar with testing.

Contact Information: Neil Boonham, Fera, Sand Hutton, YO41 1LZ, UK, Phone: +44 1904 462332, Email: neil.boonham@fera.co.uk

### **A PLANNED DEVIATION OF A CPHST-VALIDATED PROTOCOL ALLOWS FOR USE OF A HIGH-THROUGHPUT REAL-TIME MULTICYCLER FOR DETECTION OF *PHYTOPHTHORA RAMORUM***

**Elizabeth A Bush<sup>1</sup>, Geoffrey Dennis<sup>2</sup>, Vessela Mavrodiava<sup>3</sup> and Mary Ann Hansen<sup>1</sup>**

<sup>1</sup>Virginia Tech, Blacksburg, VA; <sup>2</sup>USDA-APHIS-PPQ S/T-NPPLAP, Raleigh, NC; <sup>3</sup>USDA-APHIS-PPQ S/T-NPPLAP, Beltsville, MD

The Virginia Tech Plant Clinic (VTPC) upgraded real-time PCR testing to a high-throughput platform, ABI StepOnePlus™, for *Phytophthora ramorum* detection. The VTPC real-time PCR protocol incorporates a commercially available qPCR kit for ease of use, signal normalization and streamlined optimization. Technological advances have prompted NPDN and regulatory labs to seek replacement real-time platforms for the Cepheid SmartCycler®, currently used in PPQ-CPHST *P. ramorum* protocols. National Plant Pathogen Laboratory Accreditation Program (NPPLAP)-accredited labs must use CPHST protocols for regulated pathogen screening. Successful performance of a planned deviation is necessary for any modification to a CPHST protocol in order to establish comparability to the original validated protocol. The VTPC developed a modified protocol for use on the StepOnePlus™ and a study was designed by NPPLAP that would demonstrate comparability between the protocols. VTPC performed the planned deviation, using NPPLAP-manufactured reference materials for all tests, and demonstrated comparable precision, range, detection limit, and selectivity for the pathogen to the original CPHST protocol. Cooperation between PPQ and the VTPC has resulted in a new protocol for *P. ramorum* testing for NPPLAP-accredited labs that is adaptable for high-throughput testing and offers improvements in ease of use and increased assay performance. PPQ would like to highlight this successful process for engaging its member laboratories for other potential studies and for other labs interested in using the StepOnePlus™.

Contact Information: Elizabeth A. Bush, Virginia Tech, 101-G Price Hall, 170 Drillfield Dr., Blacksburg, VA 24061-0331, Phone: 540-231-8020, Email: ebush@vt.edu

### **NUCLEIC ACID-BASED DIAGNOSTICS: DOES THE TARGET SEQUENCE MATTER?**

**Grethel Y. Busot, Mohammad Arif, Shefali Dobhal and James P. Stack**

Department of Plant Pathology, Kansas State University, Manhattan, KS

Identification of microorganisms at the genus, species or pathovar/subspecies level is generally accomplished by cultural, morphological, biochemical or molecular tests or some combination of them. Pathogen identification to the strain or population level is often necessary to prevent the spread and establishment of potentially devastating pathogens. When conventional methods fail to provide a high level of confidence in pathogen identification and/or to provide confirmation for morphological and biochemical tests, a nucleic acid-based test (NAT) is often required. This is particularly true for emerging pathogens or organisms associated with recent disease outbreaks. A very

important step during the development of NAT diagnostics is target selection, which, in part, depends on the level of discrimination required: genus, species, pathovar, strain. Different regions of the pathogen genome can be targeted based on knowledge of pathogen population biology and genetics; coding or intergenic sequences, core or flexible genome, conserved or variable regions, and chromosomal, plasmid or virus. In some diagnostic tests, the absence or presence of the target sequence is adequate, while in other diagnostic tests the sequence of the amplified region must be determined for accurate identification. The durability of NAT diagnostics is often related to the careful selection of the appropriate target.

Contact Information: Grethel Y. Busot, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, Phone: 785-532-1380, Email: gbusot@ksu.edu

## **PARTNERING WITH THE STATE SOYBEAN COMMODITY BOARD TO PROMOTE DIAGNOSIS AND MANAGEMENT OF SCN IN SOUTH DAKOTA**

**E. Byamukama, C. Tande, K. Acharya**

South Dakota State University, Brookings, SD

Soybean is the second most important crop in South Dakota accounting for over \$2 billion in direct revenue in 2014. One of the major constraints to soybean production in South Dakota and generally in the U.S. is the soybean cyst nematode (SCN). SCN was first found in 1995 in two counties in South Dakota. SCN has since been expanding through the soybean growing counties within the state. To date, SCN is now found in 29 counties. Detection and monitoring of SCN populations in fields is important in two ways: determining the presence of SCN would indicate the need to start applying management practices and secondly, determining the SCN population density may indicate if the SCN management programs are reducing SCN numbers and whether a change in management practices is necessary. The South Dakota State University Diagnostic Clinic has partnered with the South Dakota Soybean Research and Promotion Council to create awareness of SCN in South Dakota by encouraging producers to submit soil samples for SCN diagnosis. The council sponsors SCN diagnosis, making it free to producers. Along with the report showing the SCN numbers, the producer also receives management recommendations. Because of this partnership, there has been an increase in SCN soil samples submitted to the clinic as well as increased awareness of SCN in the state. This form of relationship could be expanded to other crops and pests to encourage monitoring of the occurrences and changes in pest populations across years.

Contact Information: Emmanuel Byamukama, South Dakota State University, SPSB Box 2108, Brookings, SD 57006, Phone: 605-688-4521, Email: Emmanuel.byamukama@sdstate.edu

## **ONE NAME FOR FUNGI: EFFORTS TOWARD THE STANDARDIZATION OF THE NAMES OF PLANT PATHOGENS**

*W. Cavan Allen*<sup>1,2</sup>, *Barbara Shew*<sup>1</sup>, ***Lisa A. Castlebury***<sup>2</sup>, *Eileen M. Luke*<sup>3</sup>, *Michael A. Hill*<sup>3</sup>, *Joe F. Hegarty*<sup>3</sup>, *John M. McKemy*<sup>4</sup>, *Megan K. Romberg*<sup>4</sup>, *Betsy Randall-Schadel*<sup>5</sup>, *Nancy F. Gregory*<sup>6</sup>, *Marc A. Cubeta*<sup>1</sup>

<sup>1</sup>North Carolina State University, Raleigh, NC; <sup>2</sup>USDA Agricultural Research Service, Beltsville, MD; <sup>3</sup>Purdue University, West Lafayette, IN; <sup>4</sup>USDA Animal and Plant Health Inspection Service, Beltsville, MD; <sup>5</sup>USDA Animal and Plant Health Inspection Service, Raleigh, NC; <sup>6</sup>University of Delaware, Newark, DE

In the past, multiple names representing different life stages were often used for plant pathogenic fungi, sometimes causing confusion for regulatory officials as well as plant pathologists. However, the International Code of Nomenclature (ICN) for algae, fungi, and plants no longer allows for the use of dual nomenclature for anamorph and teleomorph stages, requiring the use of a single name for all fungal species. As a result of this change, a review has been underway for the last three years to determine the correct names for plant pathogenic fungi contained in the NPDR National Repository databases maintained by the Center for Environmental and Regulatory Information Systems (CERIS) at Purdue University. Correct names are being determined and conflicts resolved using the most recent scientific literature as maintained by the USDA ARS Systematic Mycology and Microbiology Lab (SMML), Mycobank, and various other sources. Overall, more than 10,000 species names in the SMML nomenclature database have been reviewed and updated. Additionally more than 2,000 entries were added or verified for newly described type specimens of fungal pathogens, published first reports, and specimens intercepted at ports of entry. These

data have been provided to CERIS for use in their systems to insure commonality for all NPDN users. CERIS staff have modified database systems so that NPDN lab management software can be adapted to use the new naming conventions, allowing for continued access to previous names and insuring continuity with previous work.

Contact Information: Lisa A. Castlebury, USDA Agricultural Research Service, 10300 Baltimore Ave., Beltsville, MD 20705, Phone: 301-504-5270, Email: lisa.castlebury@ars.usda.gov

## MARYLAND INVASIVE TRAINING AND OUTREACH PROGRAMS

**David L. Clement<sup>1</sup>, Mary Kay Malinoski<sup>1</sup>, and Karen K. Rane<sup>2</sup>**

<sup>1</sup>University of Maryland Extension Home and Garden Information Center, Ellicott City, MD 21042; <sup>2</sup>Plant Diagnostic Laboratory, University of Maryland, College Park, MD

Educational training and outreach programs on identifying new pests have been the focus of Maryland Extension invasive training programs for NPDN and the Sentinel Plant Network (SPN). Maryland is a diverse state in geography with plant species (both southern and northern native flora) and therefore susceptible to invasive pest introductions from northern and southern sources from interstate transport of goods as well as coastal ports, rail lines and airports. Training efforts have included hands-on workshops, development of smart phone and tablet apps, invasive pest posters, and ID card sets for extension, federal and state personnel, master gardeners and green industry audiences. Presentations have included information on emerald ash borer, Asian longhorned beetle, lantern fly, exotic bark beetles, Sirex woodwasp, Japanese longhorned beetle, boxwood blight, thousand cankers disease, oak wilt, chrysanthemum white rust, *Ralstonia solanacearum*, and *Phytophthora ramorum*.

Contact Information: David L. Clement, University of Maryland Extension, Home and Garden Information Center, 12005 Homewood Rd., Ellicott City, MD 21042, Phone: 410-531-5558, Email: clement@umd.edu

## BEYOND 'NATIONAL': THE NPDN AT WORK ABROAD

**Tom Creswell<sup>1</sup>, Carrie Harmon<sup>2</sup>, Margaret Williamson<sup>3</sup>, Robert Wick<sup>4</sup>, Robert E. Marra<sup>5</sup>, Ann Hazelrigg<sup>6</sup>, Lina Rodriguez-Salamanca<sup>7</sup>**

<sup>1</sup>Purdue University, West Lafayette, IN; <sup>2</sup>University of Florida, Gainesville, FL; <sup>3</sup>Clemson University, Clemson, SC; <sup>4</sup>University of Massachusetts, Amherst, MA; <sup>5</sup>Connecticut Agricultural Experiment Station, New Haven, CT; <sup>6</sup>University of Vermont, Burlington, VT; <sup>7</sup>Iowa State University, Ames, IA

One of the most frequently voiced needs among plant protection personnel in developing nations is training in plant disease diagnostics and insect identification skills. Through a variety of channels, NPDN and APS members are helping meet those needs by working in collaboration with multiple sponsoring agencies; including private donors, VEGA Alliance, Farmer-to-Farmer and USDA/Foreign Agriculture Service. Diagnosticians and others have provided training to dozens of participants from more than 15 countries in the Caribbean, Central and South America, and Central and South Asia. Training opportunities have covered a range of topics, including full plant pathology courses, plant disease diagnostic workshops, molecular diagnostics, IPM and managing a diagnostic laboratory. Sharing what we've learned as a network benefits the host country, connects us with international colleagues and strengthens our diagnostic skills.

Contact Information: Tom Creswell, Purdue University, 915 W. State Street, West Lafayette, IN 47907, Phone: 765-494-8081, Email: creswell@purdue.edu

### ETKNET: UPDATING THE PDIS EXERCISE MODULE

**Sharon Dobesh<sup>1</sup>**, Eileen Luke<sup>2</sup>, Mike Hill<sup>2</sup>, Joe Hegarty<sup>2</sup>, Rachel McCarthy<sup>3</sup>, Carla Thomas<sup>4</sup>, Craig Guthrie<sup>5</sup>, Jeffrey Davidson<sup>5</sup>, Stephen Bullington<sup>6</sup>, Jim Stack<sup>1</sup> and Richard Hoenisch<sup>4</sup>

<sup>1</sup>Kansas State University, Manhattan, KS; <sup>2</sup>Purdue University, West Lafayette, IN; <sup>3</sup>Cornell University, Ithaca, NY; <sup>4</sup>University of California-Davis, Davis, CA; <sup>5</sup>USDA-APHIS-PPQ, Frederick, MD; <sup>6</sup>USDA-APHIS-PPQ, Beltsville, MD

Since 2004, the NPDN has been running exercises in cooperation with the states and APHIS. The purpose of running these exercises is to train NPDN diagnosticians and appropriate associated staff in the process of chain of custody and notification of significant harmful pests, diseases and weeds. The IT system used has been PDIS 1.0 which was developed and run at Kansas State University for over ten years. This system has not been updated in many years and now has incompatibilities with software upgrades and development tools. In order to resume smooth functions of the exercise module, it was decided to use ETKnet, which was developed at the University of Florida but never implemented due to insufficient resources, as an initial start with its fundamental design. Kansas State University and Purdue University requested Farm Bill funding to upgrade the current Exercise Scenario software from the PDIS version 1.0 to the 'new' ETKnet system maintaining functionality, flexibility and ease of maintenance. Current efforts include assessing the ETKnet system developed at Florida, and determining how to move it forward as a viable and efficient tool. The Exercise Standard Operating Procedure (SOP), is also being updated to reflect current practices in an initial new pest find and will be implemented in ETKnet production and testing. The NPDN Exercise Committee is involved in the SOP revisions and will be involved in the future testing of ETKnet as processes become available.

Contact Information: Sharon Dobesh, Kansas State University, Department of Plant Pathology, 1712 Claflin Road, Manhattan, KS 66506, Phone: 785-532-1340, E-mail: sdobesh@ksu.edu

### ESSENTIALS OF MOLECULAR DIAGNOSTIC PROTOCOL VALIDATION TO DEVELOP RELIABLE AND ACCURATE DIAGNOSTICS

**Shefali Dobhal**, Mohammad Arif, Grethel Y. Busot, and James P. Stack

Department of Plant Pathology, Kansas State University, Manhattan, KS

Diagnostic methods are extremely important in disease management and surveillance. Accurate diagnostics are essential to prevent the introduction of pathogens into a region or country, to restrict pathogen spread, and to facilitate risk analysis. Therefore, diagnostic protocols should be validated and robust. Identification of a pathogen based on morphological characteristics requires taxon-specific expertise. Molecular diagnostic protocols (e.g., PCR and isothermal techniques) are accurate, rapid, and easy to perform. Development of accurate and reliable diagnostics depends on the steps used for diagnostic protocol validation. The criteria for assay development and validation include: the purpose of the assay and method selection, population biology of the pathogen, target gene selection, primer/probe design with precise thermodynamic parameters, optimization, standardization, repeatability/reproducibility (multi-lab and multi-operator validation), sensitivity, specificity (inclusivity and exclusivity panel selection), accuracy/reliability (inclusion of internal controls) and fitness of the purpose. Each step in diagnostic protocol development is critical to the accuracy, reliability, and longevity of the protocol. For example, knowledge about the population biology of the target pathogen helps to identify candidate target genes for primer/probe design and also to identify organisms for the inclusivity and exclusivity panels. In this poster we demonstrate the importance of each step to develop reliable diagnostic protocols.

Contact Information: Shefali Dobhal, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, Phone: 785-532-1380, Email: shefali@ksu.edu

### THE UNIVERSITY OF PUERTO RICO PLANT DISEASE AND INSECT CLINIC

**Consuelo Estevez de Jensen**, Irma Cabrera Asencio, Alejandro Segarra Carmona and Olga Gonzalez Cardona

Agro-Environmental Sciences, University of Puerto Rico, Juana Diaz Experiment Station, PR

The Plant Disease and Insect Clinic (PDIC) in Juana Diaz Experiment Station, Puerto Rico provides diagnosis services to growers, researchers, seed companies and the general public. The PDIC is part of the Southern Plant Diagnostic

Network (SPDN) and since 2006 has processed more than 6,000 samples. The aim of the PDIC is to provide fast and accurate diagnosis and recommendations for pest and disease management. Growers and homeowners have benefited from pest and disease diagnosis that included more than 500 visits to farms and gardens. Ten first disease and fifteen first insect reports have been published in the past seven years. A survey of Citrus Greening in citrus nurseries and orchards resulted in the identification of 'Candidatus' *Liberibacter asiaticus* in 2009 in Valencia orange. An important contribution of the PDIC is the establishment of a program for the production of disease free citrus in Puerto Rico. Other highlights of the PDIC include the identification of Asian Soybean Rust in 2011, affecting soybean winter nurseries. In 2012, bacterial wilt of tomato caused by *Ralstonia solanacearum* biovar 1 was identified in field tomatoes. In 2013, an outbreak of *Tomato chlorotic spot virus* transmitted by *Frankliniella schultzei* was diagnosed in tomatoes, peppers and lettuce. New diseases in 2014 include papaya black spot caused by *Asperisporium caricae* and bacterial canker of tomato caused by *Clavibacter michiganensis* subsp. *michiganensis*. Management of new and emerging pests and diseases in Puerto Rico has been fundamentally impacted through the work of the PDIC specialists.

Contact Information: Consuelo Estevez de Jensen, University of Puerto Rico, Carr 510 Km 3.2, Sabana Llana, Juana Diaz, PR 00795, Phone: 787-260-6037, Email: Consuelo.estevez@upr.edu

### NEW PATHOGEN AND INSECT DISCOVERIES FROM THE NEW MEXICO STATE UNIVERSITY PLANT DIAGNOSTIC CLINIC (2010 – 2015)

*Natalie P. Goldberg and Jason M. French*

New Mexico State University Las Cruces, NM

The New Mexico State University Plant Diagnostic Clinic (NMSU-PDC) provides diagnostic services for the state of New Mexico and serves as a support lab for the New Mexico Department of Agriculture (NMDA) and the National Plant Diagnostic Network (NPDN). The NMSU-PDC, an integrated clinic, processed over 11,000 disease, insect and plant identification samples between 2010 – 2015. The diagnostic capability of the clinic continues to improve and we now have the ability to offer advanced molecular diagnostics for new and regulatory pathogens. As a result of increased capability, we have identified several new pathogens or host-pathogen combinations, on average 5 per year, in New Mexico and the U.S. since 2010. Among the most interesting include: Bacterial Mosaic of Wheat (*Clavibacter michiganensis* subsp. *tessellarium*), Soil-borne Wheat Mosaic Virus (SBWMV), Buckeye Rot of Tomato (*Phytophthora nicotianae*), onion bulb rot (*Phytophthora nicotianae*), Eastern cottonwood trunk rot (*Phytophthora riparia*), Anthracnose of Sunflower Sprouts and Strawberry (*Colletotrichum acutatum*), Thousand Cankers Disease (*Geosmithia morbida*), Goss's Wilt of Corn (*Clavibacter michiganensis* subsp. *nebraskensis*), Phytophthora Blight of Bay Laurel (*Phytophthora tropicalis*), Late Blight of Tomato (*Phytophthora infestans*), Stem and Bulb Nematode of Garlic (*Ditylenchus dipsaci*), Pecan Bacterial Leaf Scorch and Bacterial Leaf Scorch of Shade Trees (*Xylella fastidiosa*). Several new insect pests have also been identified including: Honey Locust Agrilus (*Agrilus difficilis*), a close relative of the Emerald Ash Borer, Brown Marmorated Stink Bug (*Halyomorpha halys*), Bagrada Bug (*Bagrada hilaris*) and Spotted Wing Drosophila (*Drosophila suzukii*).

Contact Information: Natalie Goldberg, New Mexico State University, Extension Plant Sciences Department, P.O. Box 30003 MSC 3AE, Las Cruces, NM 88003, Phone: 575-646-1621, Email: ngoldber@nmsu.edu.

### PNW VEG: AN EXTENSION TEAM APPROACH TO DISEASE AND PEST DIAGNOSTICS, RESEARCH COLLABORATION, AND GROWER EDUCATION FOR THE VEGETABLE PRODUCTION INDUSTRY

*Jenny R. Glass<sup>1</sup>, Debra A. Inglis<sup>2</sup>, Lindsey J. du Toit<sup>2</sup>, Carol A. Miles<sup>2</sup>*

<sup>1</sup>Washington State University, Puyallup, WA; <sup>2</sup>Washington State University, Mount Vernon, WA

The Pacific Northwest Vegetable Extension Group (PNW VEG) was formed in 2000 by researchers at WSU Mount Vernon. The goal of the team is to serve the fresh market, processing, and vegetable seed industries of the three Pacific Northwest states, Washington, Oregon, and Idaho, through diagnosis of disease and pest problems and coordination of education and research efforts with a focus on environmentally-sound disease and pest management strategies. The team has expanded from an original 8 members to more than 25 today with expertise

covering plant pathology, horticulture, entomology, and weed science on a wide variety of vegetable crops grown in the Pacific Northwest. Members, many of whom belong to the NPDN, represent Washington State University, Oregon State University, University of Idaho, and USDA-ARS.

Team activities include monthly conference calls during each production season to discuss current growing conditions and disease and pest problems encountered. Email communication is commonly used for diagnostic assistance and research collaboration. Team members work together to plan research projects, write extension bulletins and research papers, and develop workshops and resources for grower education. The team maintains a comprehensive website at [http://mtvernon.wsu.edu/path\\_team/vegpath\\_team.htm](http://mtvernon.wsu.edu/path_team/vegpath_team.htm), which includes a photo gallery of vegetable problems, a calendar of regional vegetable activities, links to vegetable extension publications and other online vegetable resources, and lists of regional plant diagnostic resources. The PNW VEG team has been very successful at sharing expertise, identifying newly emerging problems, and making a positive impact on growers' management of significant disease and pest issues facing Pacific Northwest vegetable industries. The shared expertise among members enhances the capacity for individual members to serve Pacific Northwest vegetable stakeholders.

Contact Information: Jenny Rebecca Glass, WSU Puyallup Plant & Insect Diagnostic Lab, 2606 W Pioneer, Puyallup WA 98371, Phone: 253-445-4582, Email: [jennyglass@wsu.edu](mailto:jennyglass@wsu.edu)

### THE ROLE OF THE CHRP PATHOLOGY GROUP IN FLORIDA

Timothy D. Riley, **Hilda D. Gomez** and Daniel J. Robl

USDA-APHIS-PPQ-Citrus Health Response Program, FL

USDA-APHIS-PPQ Pathologists are responsible for a number of activities within the Citrus Health Response Program (CHRP), including but not limited to disease identification of samples collected during survey activities on residential properties and in citrus commercial groves or inspections of citrus nursery stock, and packinghouse fruit. Diseases of regulatory concern in Florida are citrus canker, Huanglongbing, citrus black spot, and sweet orange scab. Other exotic diseases such as citrus leprosis and citrus variegated chlorosis are also part of the multi-pest survey (MPS). Since October 2005, a total of 12,976 plant samples have been screened by pathologists for initial identification with final confirmation provided by an USDA APHIS diagnostic laboratory according to the protocols outlined in the New Pest Response Guideline for each targeted disease. To maintain field survey and inspection skills for CHRP employees, training programs have been developed for citrus disease and variety identification, digital imagery, and sample collection and submission. Together all skills are tested annually to determine the level of proficiency achieved by each field technician. APHIS-PPQ CHRP pathologists also assist in the development and implementation of field survey methodologies. The MPS risk-based model is being utilized and adjusted annually based on risk factors that increase the likelihood of finding the targeted pest. The pathologists today have a significant role in contributing to survey protocols and maintaining a chain of custody for suspect samples collected during surveys. In all, the pathology team has developed a systematic approach to pest detection from personnel training and specimen diagnosis to development of survey methodologies and implementation.

Contact Information: Timothy D. Riley, USDA APHIS PPQ CHRP, 10806 Palmbay Dr., Orlando, FL 32824, Phone: 407-851-5640, Email: [timothy.riley@aphis.usda.gov](mailto:timothy.riley@aphis.usda.gov)

### DEVELOPMENT OF A FIELD DIAGNOSTIC KIT FOR DETECTION OF WHEAT STREAK MOSAIC VIRUS

Mary Burrows<sup>1</sup>, Mark Young<sup>1</sup>, Susan Brumfield<sup>1</sup> and **Eva Grimme<sup>1</sup>**

<sup>1</sup>Montana State University, Bozeman, MT

The diagnosis of plant viral diseases in the field is a difficult task and is principally based on visual symptoms. Accurate plant viral disease diagnostics requires expertise, the use of specialized equipment, and takes days to complete. *Wheat streak mosaic virus* (WSMV) infection of small grains in Montana has resulted in significant losses for wheat growers. In recent years, disease pressure has increased due to widespread hail events near crop maturity, volunteer due to stem cutting insects such as the wheat stem sawfly, early planting dates, and warm and extended

fall temperatures. In 2014, 46 samples of WSMV were submitted to the Schutter Diagnostic Laboratory and in 2015, 89 samples. Disease monitoring plots indicate that the disease potential is high in the eastern and central winter wheat growing areas of Montana for the 2015–2016 crop year. Early and accurate identification of WSMV can aid decision making for disease management. In 2014–2015, we developed and field tested a prototype immunostrip for WSMV. Kits were distributed to extension agents, consultants, and were used in the Schutter Diagnostic Laboratory. The immunostrip was well received, providing accurate results within 1–15 minutes in the laboratory or field. The assay is now being developed for low-cost, rapid construction with 3D printing technology. We anticipate the distribution of 10,000 immunostrips for the 2016 crop year across the Great Plains of the United States and Canada. This effort aids decision making, increases the authoritativeness of the consulting agent, and increases awareness of virus disease issues by growers.

Contact Information: Mary Burrows, Montana State University, Department of Plant Sciences and Plant Pathology, 119 Plant BioScience Building, Bozeman, MT 59717, Phone: 406-994-7766, Email: mburrows@montana.edu

## VIRGINIA AGENCIES AND STAKEHOLDERS COLLABORATE TO ADDRESS AN EMERGING DISEASE

**Mary Ann Hansen<sup>1</sup>, Elizabeth Bush<sup>1</sup>, Norm Dart<sup>2</sup>, Adria Bordas<sup>3</sup>, Anton Baudoin<sup>1</sup>, Larry Nichols<sup>2</sup>, Debra Martin<sup>2</sup>, Chuan Hong<sup>4</sup>, T. Michael Likins<sup>3</sup>, and Herve Avenot<sup>1</sup>.**

<sup>1</sup>Virginia Tech, Blacksburg, VA; <sup>2</sup>Virginia Department of Agriculture and Consumer Services, Richmond, VA; <sup>3</sup>Virginia Cooperative Extension, VA; <sup>4</sup>Hampton Roads Agricultural Research and Extension Center, Virginia Beach, VA

Boxwood is a significant component of historic and contemporary Virginia landscapes, and an important agricultural commodity in Virginia. Virginia also has a substantial boxwood greenery industry, which provides holiday greenery to the entire East Coast of the US. Following the first detection of boxwood blight, caused by *Calonectria pseudonaviculata*, by the Virginia Department of Agriculture and Consumer Services (VDACS) in Virginia in Fall 2011, VDACS quickly assembled a task force of Virginia Cooperative Extension, research and regulatory personnel to address the problem. Initial efforts focused on containing the disease in the county of introduction, but task force members realized that a pro-active approach to disease management and a broader educational effort were needed. Task force members drafted best management practices (BMP's) tailored to individual stakeholder groups, and then met with stakeholders to provide an opportunity for them to critique the proposed best management practices. Unique perspectives and valuable insights provided by stakeholders resulted in important changes to BMP drafts. For increased visibility and ease of access, a web site was created to house BMP's, an image gallery and research-based recommendations on sanitizers, fungicides and resistant cultivars. A wallet-sized, laminated card with images of symptoms and a QR code to link to the web site was developed for distribution at Extension meetings. Feedback from growers and colleagues and downloads of best management practices from the web site indicate that the educational effort has been impactful. This collaborative effort will serve as a model for addressing future emerging diseases in Virginia.

Contact Information: Mary Ann Hansen, Department of Plant Pathology, Physiology and Weed Science, 170 Drillfield Dr., Blacksburg, VA 24061, Phone: 540-231-6530; Email: maryannah@vt.edu.

## MOLECULAR DIAGNOSTICS FOR MONILINIA SPECIES OF REGULATORY CONCERN

**Snezana Haymes, Kurt A. Zeller, Leandra Knight and Z. Gloria Abad**

USDA-APHIS-PPQ, CPHST Beltsville Laboratory, Beltsville, MD

Brown rot, caused by several *Monilinia* species, is an extremely destructive fungal disease of pome and stone fruits, having an important economic impact worldwide. Symptoms include blossom, twig and leaf blights, stem cankers, and fruit rots. *Monilinia fructicola* and *M. laxa* are common pathogens in the United States while *M. fructigena* and *M. polystroma* have not yet been detected in the country, but could be introduced from either Europe or Asia. These two species are listed as priority pests in the Cooperative Agricultural Pest Survey (CAPS) Lists of Pests of Economic and Environmental Importance (2014–2016). *Monilinia mumecola* and *M. yunnanensis* are two other fruit rot causing species of less known impact that have been described recently from Japan and China. Identification of *Monilinia* spp. based on morphological characteristics is difficult and no validated molecular methods are currently available

for use in CAPS surveys. Being able to differentiate clearly among these species would be of benefit to PPQ, and to our partner State and NPDN laboratories. In this work, we assessed a conventional PCR assay for specific detection of *M. fructigena* and *M. polystroma* against a collection of *Monilinia* spp. isolates that included representatives of six *Monilinia* species from nine different hosts. Our preliminary results indicate that the assay allows for specific detection of *M. fructigena* and *M. polystroma*. Results of further verification of this assay, including the sensitivity and specificity testing, will be presented.

Contact Information: Snezana Haymes, USDA-APHIS-CPHST, BARC-East, Bldg. 580, Powder Mill Rd., Beltsville, MD 20705, Phone: 301-313-9233, Email: snezana.haymes@aphis.usda.gov

### PROMOTING IPM THROUGH A PARTNERSHIP BETWEEN THE UVM PLANT DIAGNOSTIC CLINIC AND THE VERMONT EXTENSION MASTER GARDENER PROGRAM

*Ann Hazelrigg and Gabriella Maia*

University of Vermont, Burlington, VT

Gardeners often seek out pest and disease identification and management advice from untrained staff at local garden centers and big box stores. In these cases, pesticides may be recommended without ever seeing the ‘pest’ or getting a positive diagnosis. In Vermont, gardeners can contact the toll free UVM Extension Master Gardener Helpline to discuss the pest or disease problem with a trained Master Gardener. When a positive diagnosis of the problem is warranted, the gardener is encouraged to send a sample to the UVM Plant Diagnostic Clinic for identification and management options. Samples are processed and problems are diagnosed within a 48 hour period. Control options that includes lifecycle information and IPM strategies are discussed with the gardener and the Master Gardener Helpline staff. Through this partnership, the gardener is less likely to resort to using a pesticide to manage the pest or disease problem and the Helpline staff receives further training on specific pests and diseases and their management.

Contact Information: Ann Hazelrigg, University of Vermont, 63 Carrigan Dr., Burlington, VT 05405, Phone: 802-656-0493, Email: ann.hazelrigg@uvm.edu

### NPDN IT COMMITTEE: THE NPDN WOULDN'T BE POSSIBLE WITHOUT IT

*Eileen Luke<sup>1</sup>, Sherri Clark<sup>2</sup>, Judy Dizon<sup>3</sup>, Martin Draper<sup>4</sup>, Lee Duynslager<sup>5</sup>, Joseph Hegarty<sup>1</sup>, Michael Hill<sup>1</sup>, Dawei Jia<sup>6</sup>, Todd Powell<sup>7</sup>, Carla Thomas<sup>8</sup>, John VanDyk<sup>9</sup>, Mike Vysocka<sup>10</sup>, Jiannong Xin<sup>6</sup>, Serena Yang<sup>6</sup>*

<sup>1</sup>CERIS - Purdue University, West Lafayette, IN; <sup>2</sup>University of Georgia, Athens, GA; <sup>3</sup>Kansas State University, Manhattan, KS; <sup>4</sup>USDA NIFA, Washington, DC; <sup>5</sup>Michigan State University, East Lansing, MI; <sup>6</sup>University of Florida, Gainesville, FL; <sup>7</sup>PClinic, Clarksburg, MD; <sup>8</sup>University of California, Davis, CA; <sup>9</sup>Iowa State University, Ames, IA; <sup>10</sup>North Carolina State University, Raleigh, NC

How many things in this world can claim to be both Glue and Grease? Information Technology (IT) can!

IT facilitates the sharing of diagnostic data from around the country, from a variety of different Lab Information Management Systems (LIMS), and **glues** it all together in the National Data Repository (NDR). The robust and responsive NDR allows diagnosticians, epidemiologists, and policy makers to aggregate, analyze, and report on collected information. The development of new and improved mapping and charting capabilities in the NDR will further enhance users' ability to understand data trends, and create understandable, compelling visuals.

All the while, IT exists in the trenches as LIMS, and most laboratories can't imagine doing business without them. Whether it's the ability to enter sample data easily as it's being worked on, locate critical information quickly, accommodate lab-specific requirements, or support features like remote sample submission and image management, LIMS **grease** sample submission operations within the lab.

The IT Committee supports both of these concepts, while making sure the processes is secure, reliable, and available. And as technology evolves, the IT Committee will continue to investigate and incorporate innovative solutions to improve users' overall experience.

Contact Information: Michael Hill, CERIS - Purdue University, 1435 Win Hentschel Blvd., Suite 207, West Lafayette, IN 47906, Phone: 765-494-9854, Email: mikehill@purdue.edu

**WPDN MALACOLOGICAL WORKSHOPS & TRAINING AND EDUCATION TRAINING AND CONNECTING IN THE WEST**

**Richard W. Hoenisch, Carla Thomas, and Richard M. Bostock**

Western Plant Diagnostic Network, University of California Davis, Davis, CA

The Western Plant Diagnostic Network (WPDN) Training & Education team organizes identification workshops, First Detector (FD) training workshops, and publishes quarterly newsletters with a distribution of over 6,200. The WPDN, in conjunction with the NPDN Training and Education team, continually develops resources for First Detectors, educators and others that increase the likelihood of early detection and identification of unknown or high-consequence plant pests and pathogens. In March, 2013, and again in June, 2015, the WPDN organized workshops for training federal, state, and county identifiers in invasive snails and slugs. Invasive snails and slugs cost the United States billions of dollars annually and early detection reduces the impact on our food supply, natural environments and the economy. The 2015 Invasive Snail and Slug workshop was videographed in its entirety and is available on the WPDN website. Included in this Snail and Slug section are all the malacological literature and guides for identification. The WPDN Training and Education program has developed and continues to update many Power Point presentations on specific invasive pests. The program has translated many of these into Spanish. These PPT resources are all found online on the WPDN site under “Presentations.”

The WPDN Training and Education team has trained over 5,500 First Detectors. The WPDN remains in contact with these FDs with the quarterly WPDN newsletters, pest alerts, and updates.

Contact Information: Richard W. Hoenisch, Western Plant Diagnostic Network, Department of Plant Pathology, University of California, Davis, One Shields Avenue, Davis, CA 95616, Phone: 530-219-2555, Email: [rwhoenisch@ucdavis.edu](mailto:rwhoenisch@ucdavis.edu)

**BACTERIAL BLIGHT/ANGULAR LEAF SPOT OF COTTON CAUSED BY XANTHOMONAS CITRI PV. MALVACEARUM IN NORTH FLORIDA**

**FB Iriarte<sup>1</sup>, S Timilsina<sup>2</sup>, P Zhang<sup>2</sup>, MJ Mulvaney<sup>3</sup>, JB Jones<sup>2</sup>, NS Dufault<sup>2</sup>, GE Vallad<sup>4</sup>, SM Olson<sup>1</sup>, JJ Marois<sup>1</sup>, DL Wright<sup>1</sup>, ML Paret<sup>1</sup> and EE Silva<sup>1</sup>**

<sup>1</sup>University of Florida, IFAS North Florida Research and Education Center, Quincy, FL; <sup>2</sup>University of Florida, IFAS Gainesville, FL; <sup>3</sup>University of Florida, IFAS West Florida Research and Education Center, Jay FL; <sup>4</sup>University of Florida, IFAS Gulf Coast Research and Education Center, Wimauma FL

Bacterial blight also known as angular leaf spot is an important bacterial disease of cotton (*Gossypium hirsutum* L.). Even though outbreaks of this disease are infrequent, serious epidemics can occur under favorable environmental conditions wherever susceptible cultivars are grown. Bacterial blight was historically a major cotton disease across the U.S. Corn Belt. The disease is caused by the bacterium *Xanthomonas axonopodis* pv. *malvacearum* (Syn: *Xanthomonas campestris* pv. *malvacearum*). Resurgence of this disease occurred in FL late July 2015 with angular leaf spot lesions observed on cotton cv. Deltapine 1137 and PhytoGen 499 in Jay, Santa Rosa County, Florida. The disease also has been reported in recent years in MS (2011–2012), AR (2011), MO (2012), and LA (2014). Symptoms of the disease included water-soaked angular leaf spots with a red to brown border. Dark yellow pigmented bacteria were consistently isolated from leaf spot lesions. Multilocus sequence analyses based on five housekeeping genes (*fusA*, *gapA*, *gltA*, *gyrB* and *lacF*) were performed. Phylogenetic trees that included 42 *Xanthomonas* species, grouped this new strain together with *X. citri* subsp. *malvacearum* (ICPB 10531 and ATCC 49290). Koch's postulates were conducted on first open boll stage cotton cultivar DP1454NR BZRF transplanted from field to greenhouse and also on 3 months old DP 1137 and PHY 499. Plants were inoculated with 10<sup>7</sup> cfu/ml suspension of the new bacterial strain. Five days later vein necrosis and leaf marginal necrosis were observed. The same bacterial pathogen was re-isolated from leaf lesions. No disease symptoms were detected in plants inoculated with water.

Contact Information: Fanny Iriarte, UF NFREC 155 Research Road, Quincy, FL 32351, Phone: 850-875-7140, Email: [fbiriarte@ufl.edu](mailto:fbiriarte@ufl.edu)

## SPIDER DIAGNOSTICS: REDUCING FEARS AND MYTHS

**Laurie Kerzicnik**

Montana State University, Bozeman, MT

Despite their negative reputation, spiders are essential members of the global ecosystem. They are present in all terrestrial habitats, except Antarctica, and thrive wherever insect prey and vegetation are present. There are only three groups of spiders that can cause real harm to humans in the United States: black widows (*Latrodectus hesperus*, *Latrodectus mactans*, and *Latrodectus variolus*), a brown widow (*Latrodectus geometricus*), and the brown recluse (*Loxosceles reclusa*). Unfortunately, spiders often take the blame for any sort of lesion or bite that appears on someone's body. A spider bite and its associated symptoms are often based solely on anecdotal evidence without a spider being captured or professionally identified. For years, spider bites have been chronically over diagnosed. These misdiagnoses could, in actuality, be infections (bacterial, fungal, or viral), allergic reactions, other arthropod bites, or many other serious medically-related conditions. The most common cause for necrotic lesions in the West is a bacterial infection called MRSA (Methicillin Resistant *Staphylococcus aureus*) infection. In Montana, only the black widow is of medical concern to humans. Although abundant in Montana, there is no conclusive evidence that the hobo spider (*Eratigena agrestis*) venom causes necrosis in humans. Fear associated with this spider is greatly exaggerated. At the Schutter Diagnostic Lab at Montana State University, we received 235 spiders for identification in 2015. Through identifications and outreach events, misinformation is clarified, fear is reduced, and the benefits of spiders are emphasized.

Contact Information: Laurie Kerzicnik, Montana State University, 119 Plant Bioscience Bldg., Bozeman, MT 59715, Phone: 406-994-5704, Email: lauren.kerzicnik@montana.edu

## RECENTLY IDENTIFIED PLANT DISEASES IN HAWAII, THEIR IMPACTS, PATHS OF ENTRY OR ESTABLISHMENT AND CURRENT STATUSES

**Mann Ko, Bonnie Dietrich, and Janis Matsunaga**

Hawaii Department of Agriculture, Honolulu, HI

Twenty plant diseases have been identified during the past three years in Hawaii that are either new to the U.S. or Hawaii. Their host impacts varied, ranging from mild to severe. Regardless, their effects on closely-related endemic or endangered Hawaiian plant species were also monitored. The diseases were considered “new” for various reasons including: Recent introductions, extant plant pathogens infecting new hosts, detection by sensitive molecular techniques, and nomenclature changes due to recent updates in pathogen taxonomy and systematics. The statuses of their distribution since first-detection also varied, ranging from “absent” due to eradication to “widespread” because some were not timely detected and so established subsequently.

This poster discusses how the diseases were discovered, their symptoms, impacts, possible pathways of entry into Hawaii, and whether they are currently considered “eradicated” or “established.” The following host-pathogen pairs are examples to illustrate these various issues: The rusts *Pucciniastrum boehmeriae* on *Pipturus albidus*, *Phakopsora jatrophiicola* on *Jatropha integerrima*, *Phakopsora phyllanthi* on *Phyllanthus acidus*, *Coleosporium plumeriae* on *Catharanthus roseus*, and *Melampsora* sp. on *Salix* spp.; the white blister rust *Pustula centaurii* on *Centaurium erthraea*; the powdery mildews *Pseudoidium jasmini* on *Jasminum sambac* and *Leveillula taurica* on *Tropaeolum majus*; the downy mildew *Plasmopara obducens* on *Impatiens walleriana*; the anthracnose *Colletotrichum petchii* on *Dracaena* sp. and finally the Cercospora leaf spot *Pseudocercospora nymphaeacea* on *Nymphaea* sp.

Contact Information: Mann Ko, Hawaii Department of Agriculture, 1428 South King Street, Honolulu, HI 96814-2512, Phone: 808-973-9546, Email: mann.p.ko@hawaii.gov

**A NEW BUGWOOD IMAGES****Joseph LaForest**<sup>1</sup>, Terrence Walters<sup>2</sup><sup>1</sup>University of Georgia, Tifton, GA; <sup>2</sup>USDA ITP, Fort Collins, CO

The Bugwood Center (The University of Georgia, Center for Invasive Species and Ecosystem Health) and ITP (USDA APHIS) have released a significant update to the end-user interfaces for four of Bugwood's image sites (<http://images.bugwood.org/>). Forestry Images, Insect Images, IPM Images, and Weed Images have a new look with added navigation and functionalities to support sharing and the use of images.

Users are now offered powerful filtering capabilities, the ease of requesting and managing their image permissions, and the ability to develop personalized image collections for presentations, sharing, and embedding into other sites. Sites now have a responsive design to provide optimal viewing and interaction experiences across a wide range of devices including your desktop, tablet, and mobile phone.

Today, [images.bugwood.org](http://images.bugwood.org) is considered the primary source by governmental agencies, NGOs, and academic institutions for images associated with natural resources and agricultural pests, diseases, and weeds. Images from the site are used globally for a multitude of purposes including education, presentations, reporting, outreach, and publications.

Contact Information: Joseph LaForest, Center for Invasive Species and Ecosystem Health, University of Georgia, 2360 Rainwater Road, Tifton, GA 31793, Phone: 229-386-3298, Email: [laforest@uga.edu](mailto:laforest@uga.edu)

# Regional IPM Centers

Established in 2000 by the USDA Cooperative State Research, Education, and Extension Service (now the National Institute of Food and Agriculture), the Regional IPM Centers play a key role in implementing the National Road Map for Integrated Pest Management (IPM), which identifies strategic directions for IPM research, implementation and measurement for all pests, in all settings, throughout the

nation. The Regional IPM Centers encourage the development and adoption of IPM, drawing expertise from many disciplines to support effective, economical pest management practices that reduce risks to the environment and human health. IPM Centers serve as a hub where groups such as farmers, regulators, scientists, consumers, government agencies, pest control companies, and environmental organizations can share information and work together toward common goals. The Centers also complement and strengthen state IPM programs by promoting communication among programs and encouraging states to collaborate and build on each others' successes.

booth no. 12

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## FACILITATION OF INNOVATION THROUGH TECHNOLOGY

**Joseph LaForest**

Southern IPM Center, University of Georgia, Tifton, GA

The Southern IPM Center fosters the development and adoption of Integrated Pest Management in the South and encourages collaboration between all stakeholders within the Southern Region. One of its signature programs, Facilitation of Innovation Through Technology (FITT) seeks to accomplish this by providing shared tools for aggregation and distribution of information. This can be used to unite research efforts from disparate programs, provide direct flow of information from research to extension, and provide extension personnel with tools that can be integrated with existing efforts to assist in outreach. In addition, these tools include logging to provide their users with appropriate indicators for utilization, spread of information, and the potential for impact from information sharing.

Current tools focus on electronic newsletters, images, video, presentations, occurrence data, pest occurrence maps, project coordination, online communication, smartphone apps, and other forms of IPM resources. All tools are provided free of charge and are available based on demand for the tools. Any interested parties are encouraged to explore the list of available tools at <http://ipmcenters.org/ipmdelivery> and contact the program staff for assistance with specific implementations.

Contact Information: Joseph LaForest, Southern IPM Center, University of Georgia, 2360 Rainwater Road, Tifton, GA 31793, Phone: 229-386-3298, Email: [laforest@uga.edu](mailto:laforest@uga.edu)

## THE ROLE OF INQUIRY DATA IN MONITORING ECONOMICALLY IMPORTANT DISEASES – RESULTS FROM THE PDIO PLANT DISEASE INQUIRY DATABASE

**Yonghao Li and Lindsay Patrick**

The Connecticut Agricultural Experiment Station, Department of Plant Pathology and Ecology, New Haven, CT

The objectives of this study were to illustrate how plant disease databases may be used to monitor epidemics of plant diseases and to develop integrated pest management programs. We mined 106,620 plant disease and plant-related inquiries during the years of 1998 – 2014 that were compiled from the Plant Disease Information Office (PDIO) inquiry database at the Connecticut Agricultural Experiment Station. Specifically we examined trends for particular host plants, their associated pathogens and for seasonal occurrences of their epidemics. The results have highlighted three types of plant disease epidemics in Connecticut: emerging (e.g. boxwood blight, *Stigmina needlecast* of spruce), recurrent (e.g. late blight of tomato, *Canavergilla needlecast* of white pine), and annual (*Volutella canker* of boxwood, *Rhizosphaera needlecast*, *Septoria leaf spot*). We demonstrate that the PDIO plant disease inquiry database can be a useful tool to monitor the onset of economically important diseases and may be helpful in developing predictive disease management programs based on their epidemic characteristics, current weather patterns, and changes in cultural practices.

Contact Information: Yonghao Li, The Connecticut Agricultural Experiment Station, Department of Plant Pathology and Ecology, 123 Huntington St. New Haven, CT 06511; Phone: 203-974-8601; Email: [yonghao.li@ct.gov](mailto:yonghao.li@ct.gov)

## DEVELOPING CANARY ASSAYS FOR PLANT PATHOGEN DETECTION

**Zhonghua Liu<sup>1</sup>, Gang Wei<sup>2</sup>, Fran Nargi<sup>3</sup>, Zhaowei Liu<sup>2</sup>, Paule-Esther Peaker<sup>1</sup>, Gloria Abad<sup>2</sup>, John Bienapfl<sup>2</sup>, Mark K. Nakhla<sup>2</sup>**

<sup>1</sup>PathSensors, Inc., Baltimore, MD; <sup>2</sup>USDA-APHIS-PPQ-S&T-Center for Plant Health Science and Technology (CPHST), Beltsville Laboratory, Beltsville, MD; <sup>3</sup>Bioengineering Systems & Technologies, MIT Lincoln Laboratory (MIT-LL), Lexington, MA

CANARY (Cellular Analysis and Notification of Antigen Risks and Yields) is an immunological assay that utilizes engineered biosensors expressing specific antibodies on their surface. Binding to pathogenic targets triggers activation of the CANARY cells, causing them to luminesce. Multiple instrument platforms and proprietary software algorithms measure and interpret this bioluminescence response. Due to innate characteristics of the CANARY biosensors the CANARY technology is able to combine the sensitivity of PCR with the speed of lateral flow

devices. A CANARY biosensor and assay can be made to detect any target for which a monoclonal antibody can be created, including bacteria, viruses, fungi and toxins. CANARY assays are currently in development for *Ralstonia solanacearum*, *Citrus leprosis* viruses cytoplasmic type (CiLV-C1 and CiLV-C2) and *Phytophthora* spp. The *Ralstonia* assay has been field-tested with geranium at PPQ plant inspection stations and demonstrated no erroneous results; the assay can be completed within 5 minutes and received positive feedback for ease of use. The *Citrus leprosis* assay also requires less than 5 minutes to complete after sample extraction. It has been tested with sweet orange leaves obtained from Mexico and has demonstrated >98% positive and negative predictive values for CiLV-C1. It did not show cross-reactivity with CiLV-C2 or *Citrus leprosis* virus nuclear type (CiLV-N). The *Phytophthora* assay, in early development, has been used to successfully detect 11 species of *Phytophthora*. These data suggest that the CANARY technology is an attractive platform for the accurate and rapid screening for plant pathogens in an easy to use format.

Contact Information: Zhonghua Liu, PathSensors, Inc., 800 W Baltimore Street, Baltimore, MD 21201; Phone: 443-557-6150, Email: zliu@pathsensors.com

## MODEL FOR EDUCATING THE NEXT GENERATION OF PLANT DIAGNOSTICIANS

### Emma Lookabaugh

North Carolina State University, Raleigh, NC

The Plant Disease and Insect Clinic at NC State University developed a model system for training and educating graduate students in all aspects of plant disease diagnostics. Integral parts of the training experience included development of communication and leadership skills, in addition to proficiency in traditional and molecular diagnostic tools. This training experience was facilitated through a graduate student assistantship in the Plant Disease and Insect Clinic (PDIC) at NC State University. The two main components of the assistantship were diagnostic training through the PDIC and completion of a graduate research project. The graduate student appointed this assistantship completed extensive course work in basic plant pathology and mycology and received academic credit for working in the PDIC. During her time in the clinic, she gained extensive knowledge of plant disease diagnosis, including diagnostic work-up, disease and pathogen identification (culturing, microscopic examination, Biolog, ELISA, and PCR) and offering control and management recommendations. In addition, she gained a strong working knowledge of all aspects of daily clinic operations and is aware of quality management components as they relate to plant disease diagnosis. She conducted original research using several traditional diagnostic tools to isolate, identify, and characterize *Pythium* species found on floriculture crops and gained expertise in the use of sequence analysis software. She also developed an efficient method for screening isolates of *Pythium* for fungicide resistance. In addition to diagnostics and research, she gained valuable experience communicating to first responders and developed social media tools for outreach and grower education.

Contact Information: Emma Lookabaugh, NC State University, 100 Derieux Pl. Campus Box 7211, Raleigh, NC 27695, Phone: 919-515-3619, Email: eclookab@ncsu.edu

## DEVELOPMENT OF RECOMBINASE POLYMERASE AMPLIFICATION ASSAYS TO RAPIDLY DETECT PHYTOPHTHORA SPECIES ON PLANT SAMPLES

Timothy D. Miles<sup>1</sup>, Frank N. Martin<sup>2</sup>, and Michael D. Coffey<sup>3</sup>

<sup>1</sup>California State University, Monterey Bay, Seaside CA; <sup>2</sup>USDA-ARS, Salinas, CA; <sup>3</sup>University of California, Riverside, CA

Fluorometric recombinase polymerase amplification (RPA) assays for the genus *Phytophthora* have been developed that provide a simple and rapid method to detect the pathogen (*Phytopathology* 105:265-278). These assays are extremely tolerant of inhibitors present in many plant extracts, thereby simplifying DNA extraction procedures. RPA assays have been developed for *Phytophthora* genus specific detection, species-specific assays for 9 taxa including *P. ramorum* and *P. kernoviae*, and a plant internal control. Assays were validated for specificity using DNA extracted from more than 135 *Phytophthora* taxa, 22 *Pythium* spp., and several plant species with a sensitivity of detection approaching TaqMan real time PCR. The assays were validated with 250+ symptomatic plant field samples

representing more than 50 hosts. Samples that were positive using the *Phytophthora* genus specific RPA test were also positive using TaqMan PCR and traditional isolation techniques. A technique for the generation of sequencing templates from positive samples to confirm species identification also was developed. Use of species specific TaqMan probe sequences for designing species specific RPA primers provides a systematic approach for assay development. These RPA assays have benefits over PCR because they are rapid (completed in as little as 15 minutes), do not require DNA purification or extensive training to conduct, require less expensive equipment and can be completed directly in the field with portable equipment. The *Phytophthora* genus specific assay has been modified for use in a lateral flow device and is currently undergoing validation, thereby simplifying the ability to complete diagnostics in the field.

Contact Information: Frank Martin, USDA ARS, 1636 East Alisal St., Salinas, CA 93905, Phone: 831-755-2873, Email: frank.martin@ars.usda.gov

## A SYSTEMATIC APPROACH FOR DEVELOPMENT OF DIAGNOSTIC ASSAYS FOR PLANT PATHOGENIC OOMYCETES

### Frank Martin

USDA-ARS, Salinas, CA

In an effort to simplify the development of diagnostic assays using high copy number targets a mitochondrial genomics approach has been used. Over 250 mitochondrial genomes have been assembled for a range of *Phytophthora*, *Pythium*, and *Aphanomyces* spp., downy mildews, and other oomycetes. Comparative genomics has identified gene order differences and regions of high sequence divergence that are useful for assay design.

For *Phytophthora*, conserved gene order differences were targeted for diagnostic assay development, thereby increasing the specificity of the assay and reducing the importance of variation in calibration among thermal cyclers. The same approach has been used for *Pythium* with this assay currently undergoing validation. For both these genera a sequence database has been developed that provides a systematic approach for design of species specific assays. For design of species specific assays for downy mildews (a *Bremia*, *Peronospora* and *Pseudoperonospora* sp.), the most effective approach has been to use unique regions as targets. This approach is currently being used for design of a diagnostic assay for *Peronosclerospora philippinensis* as part of a broader project with this pathogen.

Comparative genomics has been useful for identification of intraspecific polymorphisms that are useful as markers for population analysis and monitoring distribution of clonal lineages. It also has identified loci that are useful for species identification or phylogenetic analysis and conserved regions for design of primer annealing sites that will work across a broad spectrum of taxa. This is particularly useful for downy mildews due to the challenges of obtaining purified DNA.

Contact Information: Frank Martin, USDA ARS, 1636 East Alisal St., Salinas, CA 93905, Phone: 831-755-2873, Email: frank.martin@ars.usda.gov

## SYSTEMATIC DEVELOPMENT OF MARKERS FOR PHYTOPHTHORA SPECIES USING MITOCHONDRIAL LOCI

Timothy D. Miles<sup>1</sup>, Gregg Robideau<sup>2</sup>, Frank N. Martin<sup>3</sup>, Guillaume Bilodeau<sup>2</sup> and Mike Coffey<sup>4</sup>

<sup>1</sup>California State University-Monterey Bay, CA; <sup>2</sup>Canadian Food Inspection Agency, Ontario, Canada; <sup>3</sup>USDA-ARS, Salinas, CA;

<sup>4</sup>University of California, Riverside, CA

A number of *Phytophthora* species are non-native to the United States and are considered potentially devastating if introduced through shipment of plant material. A TaqMan real time PCR genus and species-specific diagnostic assay was developed for their detection based on mitochondrial gene order differences that allows for the systematic development of species-specific TaqMan probes. Previous research validated this marker system for 14 *Phytophthora* spp. (*Phytopathology* 104: 733–748) and the project was recently expanded by designing over 100 *in silico* species-specific probes as well as optimizing the system by normalizing annealing temperature and probe concentration across all assays. In addition, 36 new species-specific probes targeting primarily invasive species were validated against 135 different taxa in two laboratories. All probes were found to be species-specific and could be multiplexed with a genus-specific probe and a plant internal control. The assays were also validated with multiple thermal cyclers in two labs to identify potential problems with technology transfer. In an effort to simplify identification of multiple species present in a single sample, RFLP and terminal RFLP fragment analysis (primers were fluorescently

labeled and fragment sizes determined on a DNA sequencer) of the genus specific amplicon digested with several restriction enzymes was conducted. To facilitate isolate identification in these mixed samples a Java based program was developed. This system represents a comprehensive, hierarchical approach to increase detection capability and provide important tools for investigating the community structure of *Phytophthora*.

Contact Information: Frank Martin, USDA ARS, 1636 East Alisal St., Salinas, CA 93905, Phone: 831-755-2873, Email: frank.martin@ars.usda.gov

## THE NPPLAP PROFICIENCY TESTING PROGRAM: TEN YEARS OF EXPANDING LABORATORY CAPACITY AND PROMOTING QUALITY FOR REGULATED PLANT PATHOGENS TESTING

**Vessela Mavrodieva<sup>1</sup>, Geoffrey Dennis<sup>2</sup> and Patrick Shiel<sup>2</sup>**

USDA APHIS PPQ CPHST <sup>1</sup>Beltsville Laboratory, Beltsville, MD and Director's Office <sup>2</sup>, Raleigh, NC

The Proficiency Testing (PT) Program of the National Plant Protection Laboratory Accreditation Program (NPPLAP) has been instrumental in achieving the NPPLAP goal of expanding the diagnostic capacity for the USDA plant health regulatory programs and establishing a state of readiness in case of emergency. Since its beginning more 10 years ago with the *Phytophthora ramorum* Provisional Approval Program, the PT Program has added two more panels for citrus huanglongbing (HLB) in 2007 and plum pox virus (PPV) in 2011 to currently serve more than 30 laboratories, most of which are NPDN or affiliated with the NPDN laboratories. More than 100 panels are distributed annually. PT panels are designed to mimic real diagnostic situations in terms of sample types, pathogen diversity and range of concentration, assay specificity and sensitivity, and result interpretation. After thorough validation of the PT materials, blind and randomized panels are assembled and distributed on a predetermined schedule to the participating laboratories. The PT staff has worked diligently to significantly improve panel composition, work instructions, panel packaging and customer communications. The mutually beneficial collaboration of the NPPLAP with NPDN and other participating laboratories has resulted in a network of laboratories better equipped to meet the challenges of providing modern plant health diagnostic services.

Contact Information: Vessela Mavrodieva, USDA APHIS PPQ CPHST, BARC-East Bldg. 580, Powder Mill Rd., Beltsville, MD 20705, Phone: 301-313-9208, Email: vessela.a.mavrodieva@aphis.usda.gov

## SENTINEL PLANT NETWORK: INCREASING EARLY DETECTIONS THROUGH TRAINING AND OUTREACH

**Rachel McCarthy<sup>1</sup>, Daniel Stern<sup>2</sup>, Mike Hill<sup>3</sup>, Marc Fuchs<sup>4</sup>, Casey Sclar<sup>2</sup>, Eileen Luke<sup>3</sup> and Marty Draper<sup>5</sup>**

<sup>1</sup>Cornell University, Ithaca, NY; <sup>2</sup>American Public Gardens Association, Kennett Square, PA; <sup>3</sup>Purdue University, West Lafayette, IN; <sup>4</sup>Cornell University, Geneva, NY; <sup>5</sup>USDA-NIFA, Washington, DC

The Sentinel Plant Network is a partnership between the National Plant Diagnostic Network and the American Public Gardens Association that promotes plant conservation through early detection and rapid response. Launched in 2010 with Farm Bill support from USDA-APHIS, this partnership merges the scientific expertise and educational resources of the NPDN with the horticultural expertise and large public draw of the public garden community and vastly expands the country's readiness to detect new plant pests and pathogens.

Since September of 2011 the Sentinel Plant Network has conducted 16 training workshops and engaged 420 individuals from 170 different public gardens and over 80 individuals from NPDN, APHIS and other stakeholder groups to work together more efficiently on the front lines of early detection. Over 200 member gardens participate in the program and have committed to routine monitoring of their collections and natural areas, and to working with diagnosticians any time they find something suspect that may require confirmation. Additionally, many gardens use Sentinel Plant Network training and outreach materials to educate their volunteers and communities about the impacts of high-consequence plant pests and encourage them to act as First Detectors. In 2015 all Sentinel Plant Network training and outreach materials were made publicly available and can be accessed at [www.SentinelPlantNetwork.org](http://www.SentinelPlantNetwork.org).

Contact Information: Rachel McCarthy, Cornell University, 412 Plant Science Building, Ithaca, NY 14853, Phone: 607-255-7871, Email: rachel.mccarthy@cornell.edu

## DEVELOPMENT OF MOLECULAR DIAGNOSTIC TOOLS FOR THE INVASIVE OOMYCETE PATHOGEN *PHYTOPHTHORA TENTACULATA*

Noah C. Luecke, Stephan Koenig and **Timothy D. Miles**

California State University, Monterey Bay, Seaside, CA

Phytophthora basal rot (PBR) of plants is a disease recently observed in Central California Native plant nurseries and restoration locations. The primary causal agent of this disease is the oomycete pathogen *Phytophthora tentaculata*. Prior to 2012, *P. tentaculata* was among the ~30 *Phytophthora* species not known to be present in the United States. Previous research had developed an isothermal diagnostic tool known as recombinase polymerase amplification (RPA), which had *Phytophthora* genus specific detection capability with results obtainable within as little as 15 minutes directly in the field without conventional DNA extraction. A *P. tentaculata* species-specific RPA assay was developed and specificity validated against pure DNA from 135 *Phytophthora* taxa. To test this technique for detection of the pathogen in PBR, 113 symptomatic samples were collected and evaluated with the new RPA assay as well as the previously validated TaqMan assay, ImmunoStrip and conventional culturing and baiting techniques. Results were similar across the various amplification platforms, with qPCR being the most sensitive in general. Finally, spatial models were created to test if elevation correlated with the presence of the pathogen. These results indicate that *P. tentaculata* is often present in low-lying areas when infected plant material is used for restoration. This information will assist in more efficient, rapid, sensitive, specific pathogen detection for management decisions in a field and nursery setting as well as understanding pathogen distribution and spread within a field.

Contact Information: Timothy Miles, California State University, Monterey Bay, Seaside, CA, Phone: 831-582-5146, Email: tmiles@csumb.edu

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Agdia Testing Services is an ISO/IEC 17025:2005 accredited laboratory and can accept samples from around the world for pathogen testing. We can assist in testing for a specific pathogen, a group of pathogens utilizing one of our standard crop screens, or a customized pathogen screen based on specific requirements. Additionally, we offer group Molecular tests designed to detect all pathogens at the genus and/or family level, allowing for the detection of uncommon pathogens listed on import and export permits.

Please stop by our booth and speak with one of our friendly staff. We are happy to answer any questions about our products/services as well as discuss possible collaborative opportunities for the future.

booth no. 9



## POLYMERASE CHAIN REACTION (PCR) MACROARRAY FOR MULTIPLEX POTATO VIRUS AND VIROID DETECTION

Christophe Debonneville<sup>1</sup>, Denise Altenbach<sup>1</sup> and, **Judit Monis**<sup>2</sup>

<sup>1</sup>BIOREBA AG, Reinach, Switzerland; <sup>2</sup>Eurofins STA Laboratories, Gilroy CA

Molecular methods are required for the detection of low titer viruses in dormant potato tubers Here we present a novel diagnostic tool allowing multiplex detection of seven potato viruses and one viroid. Namely, *Potato virus A*, *M*, *S*, *X*, *Y*, *Potato leafroll virus*, *Potato mop top virus*, and *Potato spindle tuber viroid* (PVA, PVM, PVS, PVX, PVY, PLRV, PMTV, PSTVd) can be detected in dormant tubers. This PCR-based technology was developed and validated in parallel with a novel rapid nucleic acid extraction method. The PCR macroarray analysis presents the advantages to be fast, reliable, sensitive (as sensitive as quantitative PCR), and cost effective. Furthermore the technology does not require the use of expensive equipment making it a method of choice for large scale testing. Results will be presented showing the detection of different PVY strains and the other above mentioned potato viruses from tubers with mixed infections. The validation of the method was performed with a large number of tubers issued from the Swiss potato seed certification program in collaboration with the Swiss Federal Agronomic Station (Agroscope) in Nyon.

Contact Information: Judit Monis, Eurofins STA Laboratories, 7240 Holsclaw Rd, Gilroy, CA 95020, Phone: 408-846-9964, Email: juditmonis@eurofinsus.com

## IN-FIELD DIAGNOSTICS AND PATHOGEN MANAGEMENT IN CROPS: RAPID SNP DETECTION AND ANALYSIS USING THE GENIE®III

**Nick Morant**<sup>1</sup>, Duncan Clark<sup>1</sup>, Michael Andreou<sup>1</sup>, Neil Boonham<sup>2</sup>, David Langton<sup>3</sup>

<sup>1</sup>OptiGene Limited, Horsham, UK; <sup>2</sup>Fera, Sand Hutton, UK; <sup>3</sup>Agrii, Throws Farm Technology Centre, Stebbing, UK

Septoria leaf blotch, caused by the fungus *Mycosphaerella graminicola* (*M.gr*) remains the most common and costly foliar disease in wheat and requires continual monitoring and fungicidal treatment; predominantly using azole sprays. Single nucleotide polymorphisms (SNPs) in the *M.gr*CYP-51 gene are reported to confer azole resistance to the fungus directly impacting the suitability and effectiveness of azole treatments.

OptiGene Ltd. in the UK is developing proprietary isothermal molecular diagnostic assays to run on its Genie®III instrument to rapidly detect *Mycosphaerella graminicola* and their associated azole resistant SNPs. By employing the LAMP method of DNA amplification, single base changes within the *M.gr*CYP-51 gene sequence can now be identified, with sample-to-SNP detection in less than an hour. These tests require minimal sample preparation and are easy to run. The use of highly-stable dried reagents, in combination with the battery powered hand-held Genie®III, allows real-time monitoring of samples in the field. Rapid diagnosis at the point of sampling enables the agronomist to analyse the resistance pressures within each location, permitting informed decisions to be made instantly on the choice of fungicidal treatment to achieve optimal results.

Contact Information: Nick Morant, OptiGene Limited, Unit 5 Blatchford Road, Horsham, West Sussex RH13 5QR, Phone: +44 1403 274980, Email: nick@optigene.co.uk

## NPDN DATABASE COMMITTEE; ASSURING QUALITY AND STANDARDS IN NPDN DIAGNOSTICS DATA

Karen Snover-Clift<sup>1</sup>, Tamla Blunt<sup>2</sup>, **Cynthia Music**<sup>3</sup>, Cavan Allan<sup>4</sup>, Lisa Castlebury<sup>4</sup>, Sherri Clark<sup>5</sup>, Marty Draper<sup>6</sup>, Nancy Gregory<sup>7</sup>, Darrell Hensley<sup>8</sup>, Mike Hill<sup>3</sup>, Brian Kunkel<sup>7</sup>, Joe LaForest<sup>5</sup>, Eileen Luke<sup>3</sup>, Karen Rane<sup>9</sup>, Barbara Shew<sup>10</sup>, Nancy J. Taylor<sup>11</sup>, Carla Thomas<sup>12</sup> and Melinda Sullivan<sup>13</sup>

<sup>1</sup>Cornell University, Ithaca, NY; <sup>2</sup>Colorado State University, Fort Collins, CO; <sup>3</sup>CERIS/Purdue University, West Lafayette, IN; <sup>4</sup>USDA-ARS, Beltsville, MD; <sup>5</sup>University of Georgia, Athens, GA; <sup>6</sup>NIFA, Washington, D.C.; <sup>7</sup>University of Delaware, Newark, DE; <sup>8</sup>University of Tennessee, Knoxville, TN; <sup>9</sup>University of Maryland, College Park, MD; <sup>10</sup>North Carolina State University, Raleigh, NC; <sup>11</sup>Ohio state University, Columbus, OH; <sup>12</sup>University of California, Davis, CA; <sup>13</sup>CPHST, Fort Collins, CO

Established in January of 2006 at the Second IT-Diagnosticians Meeting, the National Database Program Area Committee's primary goals continue to be the creation of guidelines that direct NPDN users on proper use of the

National Repository system and the review of existing data fields to determine if they meet current needs. The committee has been focused on improving database input through field modification, assessing newly available laboratory reports obtained directly from the National Repository, managing a review team to process user's requests for changes to the pest and host code listings and editing the existing repository codes. Over the last five years, the most significant accomplishment has been the inclusion of synonyms in the pest dictionary. As a complete, up to date and standardized set of diagnostic data, the NPDN National Repository is sought out as a valuable resource by researchers, epidemiologists and extension.

Contact Information: Cynthia Music, CERIS/Purdue University 1435 Win Hentschel Blvd., Suite 207, West Lafayette, IN 47906, Phone: 765-496-2381, Email: [clmusic@purdue.edu](mailto:clmusic@purdue.edu)

## **DETECTION AND DISCRIMINATION METHODS YOU CAN USE: VIRUS CHASERS 2008 – 2015**

*Francisco Ochoa-Corona<sup>1</sup>, Jennifer Olson<sup>2</sup>*

<sup>1</sup>Oklahoma State University, National Institute for Microbial Forensics and Food & Agricultural Biosecurity, Stillwater, OK; <sup>2</sup>Oklahoma State University, Plant Disease and Insect Diagnostic Laboratory, Stillwater, OK

Oklahoma State University, National Institute for Microbial Forensics and Food & Agricultural Biosecurity (NIMFFAB) is home and headquarter for Virus Chasers, a group of undergraduate, graduate, and post-doctoral students and collaborating scientists. Virus Chasers contributes scientific input to regulatory plant health emergencies and forensic plant pathology, and to agriculture and plant water biosecurity. Virus Chasers focuses on development and adaptation of technologies for sampling, identification of molecular genomic landmarks and signatures of value for detection and discrimination of plant viruses, other relevant pathogenic microorganisms and insects. The group also investigates implications of genetic data on taxonomic relationships, host-pathogen associations, global tracking dynamics, dispersal routes, and bio-geographic distribution. A contribution of methods published from 2008 to 2015 is presented.

Contact Information Francisco Ochoa-Corona, Oklahoma State University, 127 NRC, Stillwater, OK 74078-3003, Phone: 405-744-9946, Email: [ochoaco@okstate.edu](mailto:ochoaco@okstate.edu)

## **PICTORIAL GUIDE TO ROSE ROSETTE DISEASE SYMPTOMS**

*Jennifer D. Olson<sup>1</sup>, Mike Schnelle<sup>2</sup>, Kevin Ong<sup>3</sup>, Ellen Roundey<sup>3</sup> and David Byrne<sup>3</sup>*

<sup>1</sup>Plant Disease and Insect Diagnostic Laboratory, Dept. of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK; <sup>2</sup>Dept of Horticulture and Landscape Architecture, Oklahoma State University, Stillwater, OK; <sup>3</sup>Horticultural Sciences, Texas A&M University, College Station, TX

Roses are highly valued in nurseries and landscape plantings for their fragrance and attractive flowers. Rose rosette is a serious disease of roses and early identification of the disease is critical for successful management. This poster was developed to show the range of symptoms associated with rose rosette disease. Depending on the cultivar, the symptoms vary but may include witches' broom (rosette), proliferation of prickles (thorns), stunting, cane dieback, leaf distortion or discoloration, and blossom blight. This poster was prepared for display in county extension offices, retail centers, and nurseries to educate clients on the symptoms of rose rosette disease. Small images that are found in extension fact sheets are less useful since the images are small in size and image quality may be low. Therefore, this poster has high quality, full-color, large images that can be used to assist in rose rosette disease diagnosis. Smaller (8.5 x 11 in) sizes can be printed from <http://osufacts.okstate.edu>. All sizes (8.5 x 11, 11 x 17, 24 x 36 in) will be supplied at no cost by the lead author as funds provided by the USDA's National Institute of Food and Agriculture (NIFA), Specialty Crop Research Initiative Project "Combating Rose Rosette Disease: Short Term and Long Term Approaches" (2014-51181-22644/SCRI allow).

Contact Information: Jennifer Olson, Oklahoma State University, Dept. of Entomology and Plant Pathology, 127 Noble Research Center, Stillwater, OK 74078, Phone: 405-744-9961, Email: [jen.olson@okstate.edu](mailto:jen.olson@okstate.edu)



The Plant Diagnostic Information System (PDIS) is a system of web and database applications designed to meet the specific and diverse needs of plant diagnostic laboratory personnel and their customers. It consists of Administration, Submitter, Diagnostician and Billing modules. PDIS is currently deployed in 138 plant diagnostic laboratories in land grant institutions and state departments of agriculture facilitating services for plant disease diagnoses, plant identifications and insect/pest identifications.

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#### EMERGING DISEASES IN OREGON

Nancy K. Osterbauer<sup>1</sup>, Cynthia M. Ocamb<sup>2</sup>, and Maryna Serdani<sup>2</sup>

<sup>1</sup>Oregon Department of Agriculture, Salem, OR; <sup>2</sup>Oregon State University, Corvallis, OR

Oregon is a major producer of nursery plants and seeds, with 70% of these commodities traded domestically and internationally making these industries potential pathways for pest introductions. Recent pest detections support this concern. In 2015, foliage from lilacs (*Syringa vulgaris*) grown in a Marion County nursery showed a reduction in leaf size, leaf deformation, ring spots, and line patterns. Total nucleic acids analysis of six symptomatic plants revealed inserts that showed >99% homology with the published sequence for *Lilac Ring Mottle Virus* (LiRMV, GenBank Accession: U17391)(S. Scott, Clemson University). This is the first report of LiRMV in the US.

Three seedborne diseases, light leaf spot (LLS, *Cylindrosporium concentricum*), white leaf spot (WLS, *Pseudocercospora capsulae*), and black leg (*Phoma lingam*), were identified causing an outbreak in crucifer crops grown in western Oregon in 2014. Pure cultures for morphological identification were obtained by transferring single spores for each fungal pathogen from symptomatic tissue onto quarter-strength potato dextrose agar amended with streptomycin; sporulating structures produced on diseased tissue placed into moisture chambers were also examined microscopically. Species identification was verified using PCR and DNA sequencing. LLS was identified on *Brassica* crops and weeds growing in six counties. This is the first report of LLS in the USA. WLS was identified on *Brassica* spp. growing in five counties. This is the first report of WLS in Oregon. Blackleg was identified infecting *Brassica* spp. and *Rorippa curvisiliqua* growing in seven counties.

These detections reinforce concerns about nursery plants and seeds as pathways for pests.

Contact Information: Nancy K. Osterbauer, Oregon Department of Agriculture, 635 Capitol St NE, Salem, OR 97301, Phone: 503-986-4620, Email: nosterbauer@oda.state.or.us.

## EMERGING INSECT PEST—SPOTTED WING DROSOPHILA IN NORTH DAKOTA

Janet Knodel<sup>1</sup>, Esther McGinnis<sup>1</sup>, Harlene Hatterman-Valenti<sup>1</sup>, Kathy Wiederholt<sup>2</sup>, Charles Elhard<sup>3</sup>, Patrick Beauzay<sup>1</sup> and Jesse Ostrander<sup>1</sup>

<sup>1</sup>North Dakota State University, Fargo, ND; <sup>2</sup>North Dakota State University, Carrington Research Extension Center, Carrington, ND; <sup>3</sup>North Dakota Department of Agriculture, Fargo, ND

The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura) (Insecta: Diptera: Drosophilidae), is an emerging insect pest of small fruits grown in North Dakota. It was first discovered on cherry in 2013 in east central North Dakota, and quickly spread to 17 counties throughout the state. Unlike other vinegar flies that prefer to attack overripe and rotting fruit, the SWD lays its eggs in healthy, ripening fruits. Eggs hatch into small larvae or maggots and then larvae feed on the fruit, causing spoilage. Spotted wing drosophila commonly infests many small fruit crops (strawberries, blueberries, blackberries, raspberries) as well as stone fruit (cherries, peaches, nectarines, apricots, and plums). In addition, SWD will lay its eggs in cracked or damaged tomatoes and apples. In North Dakota, the SWD has been detected in blackberries, raspberries, tart cherries, Juneberries and grapes. It is unknown whether SWD will be a significant pest of newer or more novel specialty fruit crops of North Dakota, such as *Aronia*, blackberries, chokecherries, elderberries, haskaps or mulberries. Historically, small fruit growers in the state did not need to apply insecticides for insect pest control prior to harvest. The introduction and spread of the SWD will change how small fruits are grown in North Dakota. Given its wide host range, rapid spread and economic damage potential, it is essential that North Dakota fruit growers learn how to identify and manage this invasive insect pest.

Contact Information: Jesse Ostrander, North Dakota State University, Plant Diagnostic Lab, Dept. of Plant Pathology, Fargo, ND, Phone: 701-231-7854, Email: jesse.ostrander@ndsu.edu

## CYANOBACTERIA—AN UNDERAPPRECIATED THREAT TO AGRICULTURE?

Cynthia M. Ocamb, Allen Milligan, and Melodie L. Putnam

Oregon State University, Corvallis, OR

Surface waters of the U.S. are experiencing increased incidents of cyanobacterial blooms. Freshwaters often contain cyanobacteria (blue-green algae), and many are benign, but a number of species produce injurious toxins. Microcystin-LR is one of many toxins produced by various species of cyanobacteria and is a potent inhibitor of key regulatory enzymes in both mammals and plants. Plant seedlings can take up microcystin-LR, resulting in inhibition of development, root growth, photosynthesis, and ultimately, crop yield. The concentration of microcystin-LR (5 µg/L) studied was below the 8 µg/L limit recommended for recreational bathing. In other words, bodies of water that would not hurt swimmers could be damaging to crop plants. In 2001 Oregon began monitoring presence of potentially harmful cyanobacteria in surface waters. Over 100 human health advisories have been issued in the last seven years, but the warnings have not addressed use of the water for irrigation. In 2008, we collected tissue samples from farm fields located on the Tualatin river basin where crop plants were showing severe stunting, foliar chlorosis, necrosis, and bronzing. We confirmed the presence of 38 ng/g of microcystin-LR in a symptomatic blackcap raspberry plant. Control plant tissues were collected from nearby fields that were not irrigated with Tualatin river water, and all tested negative for the presence of microcystin-LR. It is unknown how often such incidents occur, but the potential damage to both human and plant health suggests that the threat of cyanobacterial blooms in waters used for irrigation is an under-studied phenomenon.

Contact Information: Allen Milligan, Oregon State University, Department of Botany and Plant Pathology, 2082 Cordley Hall, Corvallis, OR 97331, Phone: 541-737-5276, Email: Allen.Milligan@science.oregonstate.edu.

## GALL-ID: WEB-BASED TOOLS FOR THE RAPID IDENTIFICATION AND CHARACTERIZATION OF GALL-CAUSING PHYTOPATHOGENIC BACTERIA

Edward W. Davis II<sup>1,2</sup>, Alexandra J. Weisberg<sup>1</sup>, Javier F. Tabima<sup>1</sup>, **Melodie L. Putnam<sup>1</sup>**, Niklaus J. Grünwald<sup>1,2,3</sup> and Jeff H. Chang<sup>1,2,4</sup>

<sup>1</sup>Botany and Plant Pathology, Oregon State University, Corvallis, OR; <sup>2</sup>Molecular and Cellular Biology, Oregon State University, Corvallis, OR; <sup>3</sup>Horticultural Crops Research Laboratory, USDA-ARS, Corvallis, OR; <sup>4</sup>Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR

Understanding the population structure and genetic diversity of plant pathogens, as well as the effect of agricultural practices on pathogen evolution, are important for disease management. Advances in DNA sequencing technology and decreases in cost have contributed to greater reliance on use of 16S rDNA, *multilocus* sequence analysis (MLSA) gene sets, and sequences of whole genomes to genotype bacterial isolates. Correct analysis and interpretation of sequencing data can be difficult. Therefore we have developed a set of web-based tools, termed Gall-ID, to facilitate the identification and characterization of *phytopathogenic* bacteria, with a focus on those that cause gall diseases. These pathogens include members of *Agrobacterium* spp., *Pantoea agglomerans*, *Pseudomonas savastanoi*, and *Rhodococcus* spp. Users can compare 16S or MLSA gene sequences from an isolate against manually-curated databases for each of the above four bacterial groups, and generate a phylogenetic tree containing their isolate. Additional databases of 16S and MLSA gene sets for other bacteria important to agriculture are also available. Gall-ID also includes a tool for uploading and using whole genome sequencing reads to identify homologs of known virulence genes. Finally, Gall-ID provides downloadable software pipelines for core genome analysis (WGS Pipeline), calculation of average nucleotide identity (Auto ANI), and the generation of MLSA gene set databases (Auto MLSA). The Gall-ID tools are accessible at <http://gall-id.cgrb.oregonstate.edu>.

Contact Information: Melodie Putnam, Oregon State University, Department of Botany and Plant Pathology, 1089 Cordley Hall, 2701 SW Campus Way, Corvallis, OR 97331, Phone: 541-737-3472, Email: [putnamm@science.oregonstate.edu](mailto:putnamm@science.oregonstate.edu)

## TRACKING FUNGI THROUGH TIME AND SPACE: A CASE FOR VOUCHER SPECIMENS

**Yazmín Rivera<sup>1,2</sup>**, Megan K. Romberg<sup>1,3</sup>, Lisa Castlebury<sup>1</sup> and Jo Anne Crouch<sup>1</sup>

<sup>1</sup>USDA-ARS, Systematic Mycology and Microbiology Laboratory, Beltsville, MD; <sup>2</sup>Rutgers University, Department of Plant Biology and Pathology, New Brunswick, NJ; <sup>3</sup>USDA APHIS PPQ NIS, Beltsville, MD

Every year, new disease epidemics caused by plant pathogenic fungi threaten plant biodiversity and agricultural production worldwide. Historical specimens of plant pathogens maintained in herbaria and culture collections allow scientists to compare the morphological and molecular characteristics of fungi collected in the past with those in the present. Scientists have relied on the historical specimens housed in herbaria to document the occurrence of fungi throughout the world. With over one million specimens, the U.S. National Fungus Collections (Herbarium BPI) is one of the most valuable international resources for the documentation of fungi, especially for those associated with agriculture. The associated Fungal Databases serve as an international resource for fungus-host distributions, nomenclature and taxonomic literature. These combined resources are essential for the identification of plant pathogens intercepted at ports and often influence decisions on whether or not to initiate quarantine actions. While the Fungal Databases rely on peer-reviewed literature for continued growth, herbaria rely on mycologists and plant pathologists depositing voucher specimens. However in recent years, specimen deposits have been decreasing. Diagnosticians and field scientists who are in constant contact with fresh samples of plant pathogenic fungi could provide a valuable new influx of specimens. When reporting fungi on new hosts or from new locations, specimens and live cultures should be deposited in recognized collections to document these reports and preserve material for future scientists.

Contact Information: Yazmín Rivera, USDA-ARS Systematic Mycology and Microbiology Laboratory & Rutgers University, 10300 Baltimore Ave, Bldg. 010A Room 227, Beltsville, MD 20705, Phone: 301-504-5331, Email: [yaz.rivera@gmail.com](mailto:yaz.rivera@gmail.com)

## EXPANDING THE IMPACT OF NPDN: DIAGNOSTIC TRAINING IN COLOMBIA

**Lina Rodriguez Salamanca<sup>1</sup>** and Tom Creswell<sup>2</sup>

<sup>1</sup>Iowa State University, Ames, IA; <sup>2</sup>Purdue University, West Lafayette, IN

A diagnostic training was conducted from October 11 to 23, 2015 in Villavicencio Meta, Colombia as part of the Farmer to Farmer project, sponsored by USAID, VEGA Alliance, and Purdue University.

The Llanos region produces 13% of the tropical fruit in the country; including avocado, cacao, citrus, guava, papaya, passion fruit, pineapple, and plantain. Producers operations range from small to medium in size. Weather, especially temperature and relative humidity, is conducive to disease outbreaks that may result in yield reduction higher than in other tropical fruit production areas in Colombia.

Field sampling was conducted to teach the participants the importance of good sample collection practices for quality diagnosis. Daily curriculum and hands-on activities were delivered on the training to twelve participants from different institutions in Colombia. The participants and institutions had various degrees of diagnostic duties. Some institutions are planning to expand their services while others are working to improve their diagnostic protocols and procedures. The more advanced laboratory followed the ISO90001 for quality assurance.

The outreach from specialists in the university and agricultural research institutions in the Llanos area is difficult due to the lack of a formal extension service. However, efforts were discussed to develop online and mobile resources to support sustainable tropical fruit production, and to provide more accessible diagnostic services so growers can submit samples for accurate diagnosis that can lead to a better-informed decision-making process on their farms.

Contact Information: Lina Rodriguez Salamanca, Iowa State University, 327 Bessey Hall, Ames, IA 50011, Phone: 515-520-2441, Email: lina@iastate.edu

## SEARCHING FOR THE OAK WILT PATHOGEN, *CERATOCYSTIS FAGACEARUM*, IN NEW YORK STATE

**Emma Rosenthal**, Karen Snover-Clift and Sandra Jensen

Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY

*Ceratocystis fagacearum* is the fungal pathogen responsible for oak wilt, a devastating disease threatening oak trees across the mid-western and southern United States and into Pennsylvania. It was discovered twice in New York's Schenectady County, once in 2008 and again in 2013. While control measures were taken to prevent spread, it remained unknown whether the disease had become established elsewhere in the state. As a part of the Specialty Crops Block Grant, the Cornell University Plant Disease Diagnostic Clinic in collaboration with the New York State Departments of Environmental Conservation and Agriculture and Markets began a project to understand the scope of oak wilt infection in New York State. One goal was to allow for early detection of potential oak wilt infection; case studies have shown that early detection is key to a successful eradication. Another goal was to determine whether our protocol for molecular identification could be an effective method using isolated fungal specimens and bark tissue. If so, this would enable us to optimize the oak wilt diagnostic procedure for both accuracy and efficiency. In 2015, the Clinic received twenty-one samples taken from suspect trees from around New York State. We found that our testing procedure was effective using both branch tissue and fungal specimens. Morphological and molecular testing allowed us to confirm that none of the suspect trees were infected with oak wilt. Though all received samples were negative, our efforts will continue with hopes of early detection of any potential oak wilt infection.

Contact Information: Emma Rosenthal, Cornell University, 310 Plant Science Bldg. Ithaca, NY 14853, Phone: 607-255-7850, Email: err74@cornell.edu

stem rust on winter wheat. photo © Mary Burrows, Montana State University, Bugwood.org

## A FIRST DETECTION SUCCESS STORY: CORN TAR SPOT IDENTIFIED FOR THE FIRST TIME IN THE UNITED STATES BY COLLABORATIVE EFFORTS OF NPDN AND USDA-APHIS FUNGAL IDENTIFICATION LABS

Gail Ruhl<sup>1</sup>, Megan Romberg<sup>2</sup>, Tom Creswell<sup>1</sup>, Suzanne Bissonnette<sup>3</sup>, Diane Plewa<sup>3</sup>, Kiersten Wise<sup>1</sup>

<sup>1</sup>Purdue University; <sup>2</sup>USDA-APHIS-PPQ, - Beltsville MD; <sup>3</sup>University of Illinois

Corn tar spot, caused by *Phyllachora maydis*, was identified for the first time in the United States by the Purdue Plant and Pest Diagnostic Laboratory (PPDL) on corn samples submitted to the PPDL in early September 2015 from two counties in northwest Indiana and three counties in north-central Illinois. Infected leaves exhibited characteristic symptoms of tar spot, including oval to irregular bleached to brown lesions on leaves in which black, protruding spore-producing structures (ascomata) were formed. Lesions with numerous ascomata coalesced to cause large areas of blighted leaf tissue. Symptoms and signs of tar spot were also observed on husks. Official morphological and molecular confirmation of the causal fungus, *Phyllachora maydis*, was provided by the United States Department of Agriculture-Animal Plant Health Inspection Service in Beltsville, MD. A concerted effort to inform the public and a call for additional samples was conducted by both the PPDL and the University of Illinois Plant Clinic; these efforts led to an increased number of detections. Ten additional IL counties were confirmed by the Illinois Plant Clinic and five additional IN counties by the Purdue PPDL. It is suspected the fungus may have been introduced into fields in Indiana and Illinois in 2015 via wind-blown inoculum from Central America or Mexico. It is not known whether this fungal pathogen will overwinter in these Midwestern fields and thus coordinated survey efforts in 2016 will be necessary to provide this information for this first documented confirmation of *Phyllachora maydis* in the United States.

Contact Information: Gail Ruhl, Purdue University, 915 West State Street; West Lafayette, IN 47907-2054, Phone: 765-494-4641, Email: ruhlg@purdue.edu

## QUANTITATIVE UTILITY OF THE AMPLIFYRP® ISOTHERMAL PLATFORM

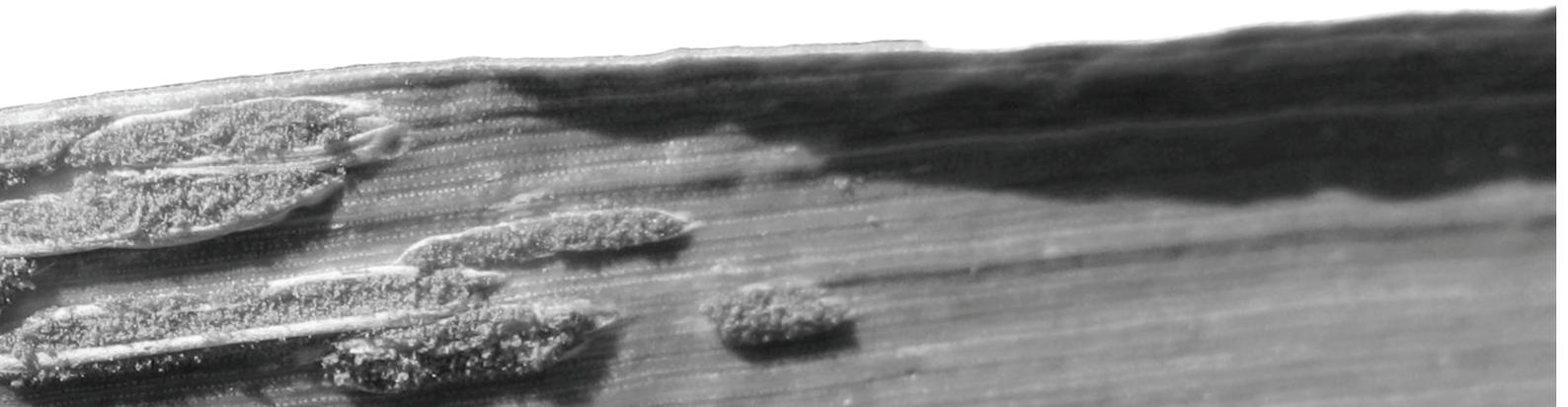
Paul F. Russel, Nathan McOwen, Bryant Davenport, Shulu Zhang, and Rugang Li

Agdia, Inc., Elkhart, IN

Molecular detection methods for plant pathogens have been advanced very quickly. The PCR-based methods were widely used in this community. However, PCR-based methods rely on the use of purified DNA or RNA as template, increasing cost and labor. In recent years, isothermal amplification has gained favor for field-test platform because of tolerance to host inhibitors and its use of a simple incubator.

The AmplifyRP® platform is an isothermal amplification system based on the recombinase polymerase amplification system. Detection of amplification can be performed in real-time using a fluorometer (XRT) similarly to realtime PCR, or as an end-point assay using lateral flow strips in an Amplicon Detection Chamber (Acceler8). Both formats are extremely sensitive and specific as qualitative assays. However, as with qPCR, the XRT has the potential to be quantitative. We present here, data from multiple tests, demonstrating that time of “onset of amplification” can be related to template concentration such that one may be predictive of the other.

Contact Information: Paul Russell, Agdia, Inc., Research & Development, 52642 County Road 1, Elkhart, IN 46514, Phone: 574-264-2014, Email: prussell@agdia.com



## **CURTOBACTERIUM FLACCUMFACIENS AND POINSETTIA: A CASE STUDY IN THE NEED FOR INFORMATIVE TAXONOMIC FRAMEWORKS**

**Maryna Serdani, Michele S. Wiseman, and Melodie L. Putnam**

Oregon State University, Corvallis, OR

Bacterial canker and leaf spot of poinsettia (*Euphorbia pulcherrima*) is caused by the bacterium *Curtobacterium flaccumfaciens* pv. *poinsettiae*. Infection results in watersoaked leaf spots and blotches, and stem infections with dark watersoaked cankers. In 2014 and 2015, we received poinsettias with symptoms of bacterial canker. Isolations from symptomatic tissues yielded yellow bacterial colonies that were identified by Biolog (MicroLog 2) variously as *C. albidum*, *C. luteum*, or *C. flaccumfaciens* pv. *flaccumfaciens*. The 16s rDNA region of five different isolates were 98–100% identical with all *C. flaccumfaciens* pv. *flaccumfaciens* accessions in GenBank, a pathogen of regulatory concern. We chose one isolate for further study. When comparing 16S rDNA of all the available *C. flaccumfaciens* type species in GenBank, our isolate showed 99% identity with *C. flaccumfaciens* pvs. *flaccumfaciens*, *poinsettiae*, and *betae*. Poinsettia cuttings inoculated with this isolate resulted in symptoms similar to those on the naturally infected plants. Inoculation to bean resulted in wilt or local stem lesions. The 16S rDNA sequencing of bacteria recovered from both inoculations was the same as above. The 16s rDNA region is known to be insufficient to identify bacteria to species or pathovar, and Biolog is problematic with Gram-positive bacteria. There is no one gene region known to be reliable for speciating *Curtobacterium* isolates, the pathovar designations for the species may be unreliable, and no taxonomic work has been done on this genus since 2000. This situation points out the paucity of meaningful taxonomic information for some genera of plant pathogenic bacteria, which is problematic for isolate identification.

Contact Information: Melodie Putnam, Oregon State University, Department of Botany and Plant Pathology, 1089 Cordley Hall, 2701 SW Campus Way, Corvallis, OR 97331, Phone: 541-737-3472, Email: putnamm@science.oregonstate.edu

## **PLANTS, PESTS AND PATHOGENS WEBINARS AT NC STATE UNIVERSITY**

**Barbara Shew, Michael Munster, Matt Bertone, and Lucy Bradley**

North Carolina State University, Raleigh, NC

One of the key elements of the training provided by the Plant Disease and Insect Clinic (PDIC) at North Carolina State University is an interactive program for Extension Agents and Master Gardener Volunteers. This program, known as “Plants, Pests, and Pathogens” (PP&P) is presented five times per year and is focused on insects and plant diseases that are currently active. Training sessions are conducted over the internet using the Blackboard Collaborate platform, which allows for images, voice, polling/quizzes, and even the division of participants into break-out groups during the program.

The usual format of PP&P consists of an opening orientation followed by a featured speaker and then 30 minutes each on diseases and arthropods of interest, including “Be on the Lookout” lists of what to expect in the upcoming months. During 2015, diagnosis was emphasized, with a corresponding change in format allowing for discussion within break-out groups. Each session featured disease and arthropod scenarios for a different group of hosts, for example, woody ornamentals. Discussion leaders, acting in the role of a client, provided information to help participants arrive at a diagnosis. The principal challenges were the extra time required for preparation of detailed scenarios, and technical delays in setting up the break-out groups. Although experienced agents dominated the conversation during some exercises, the sessions were very well received. The average participation during the 2015 sessions consisted of 14.6 agents, 27.4 MGVs logged on individually, and 32.2 MGVs connecting as part of a group.

Contact Information: Barbara Shew, Department of Plant Pathology, 100 Derieux Place, Campus Box 7616, North Carolina State University, Raleigh, NC 27695-7616, Phone: 919-515-6984, Email: bshew@ncsu.edu

## BELTSVILLE WORKSHOPS; AN NPDN AND USDA-APHIS-PPQ-S&T COLLABORATIVE EFFORT TO STRENGTHEN NATIONAL DIAGNOSTIC READINESS

**Karen Snover-Clift<sup>1</sup>**, *Vessela Mavrodieva<sup>2</sup>*, *Gloria Abad<sup>2</sup>*, *John Bienapfl<sup>2</sup>*, *John Rascoe<sup>2</sup>*, *Gang Wei<sup>2</sup>*, *Stefano Costanzo<sup>2</sup>*, *Kurt Zeller<sup>2</sup>*, *Mark Nakhla<sup>2</sup>* and *Tricia Allen<sup>1</sup>*

<sup>1</sup>Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY; <sup>2</sup>USDA-APHIS-PPQ-Center for Plant Health, Science & Technology-Beltsville Laboratory, Beltsville, MD

Since 2003, the NPDN and USDA-APHIS-PPQ-S&T staff members have worked collaboratively to provide advanced molecular diagnostic workshops on the approved and validated testing protocols for significant pathogens. The NPDN staff are responsible for seeking external funding and submitting a grant proposal, coordinating workshop participants and providing travel reimbursements. The Beltsville staff are responsible for providing trainings, including developing workshop content, producing materials (from reagents to handouts) and on-site coordination. These trainings are extremely important because participants are prepared, or could be on short notice, to provide surge capacity in the event of an unexpected outbreak of select and significant agents. The participants have also strengthened their confidence in their ability to learn new technologies and apply them as needed to protect the nation's resources. Over the 12 year period, 14 topic areas have been covered in 63 workshops to 436 NPDN and collaborating diagnosticians. The 14 topics covered included 1) soybean rust (*Phakopsora pachyrhizi* and *P. meibomia*), 2) sudden oak death (*Phytophthora ramorum*), 3) *Ralstonia solanacearum* R3B2, 4) citrus greening-HLB (*Candidatus Liberibacter asiaticus*, *Candidatus Liberibacter africanus*, and *Candidatus Liberibacter americanus*), 5) potato cyst nematode (*Globodera rostochiensis*), 6) plum pox virus, 7) *Phytophthora kernoviae* with *P. ramorum*, 8) potato wart (*Synchytrium endobioticum*), 9) *Phytophthora 101* with focus on *P. ramorum* and *P. kernoviae*, 10) bioinformatics part I, 11) bioinformatics part II, 12) citrus diseases which included Citrus Leprosis, Sweet Orange Scab (*Elsinoë australis*) and Citrus Black Spot (*Guignardia citricarpa*), 13) bioinformatics complete and 14) phytoplasmas featuring apple proliferation.

Contact Information: Karen Snover-Clift, Cornell University, 324 Plant Science Bldg. Ithaca, NY 14853, Phone: 607-255-7860, Email: kls13@cornell.edu

## STAR-D; THE CREATION AND IMPLEMENTATION OF NPDN'S LABORATORY ACCREDITATION PROGRAM FOR PLANT DIAGNOSTIC LABORATORIES

**Karen Snover-Clift<sup>1</sup>**, *Dawn Dailey O'Brien<sup>1</sup>*, *Katherine Burch<sup>2</sup>* and *Pat Shiel<sup>2</sup>*

<sup>1</sup>Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY; <sup>2</sup>USDA-APHIS-PPQ-Science & Technology, Raleigh, NC

The System for Timely, Accurate and Reliable Diagnostics (STAR-D), Laboratory Accreditation Program is the result of an effort put forth by the National Plant Diagnostic Network (NPDN) and their collaboration with the USDA-APHIS-PPQ-S&T to establish and implement a laboratory accreditation system for the NPDN's Land Grant University and State Department of Agriculture plant diagnostic facilities. STAR-D was created using the ISO-17025 national laboratory standard and the American Association of Veterinarian Laboratory Diagnosticians (AAVLD) accreditation system as models. This program is improving many aspects of early pathogen and pest detection for the network members by establishing requirements and standards. The goal is to have STAR-D accredited diagnostic labs recognized by their clients as suppliers of superior lab services and as fully responsive to urgent sample surges. To attain that goal, trainings — Quality Management System (QMS) Workshops and Phase 2-Internal Auditor Workshops — have been utilized and developed to inform and guide the membership in implementing STAR-D in their laboratories. Additionally, two ISO-17025 Auditor Trainings were provided to members who will serve as External Auditors. The first laboratories were granted accreditation status May 2014 and to date three laboratories have gained accreditation with an additional seven laboratories completing the preliminary step of a gap audit. Implementation of STAR-D among NPDN laboratories is well underway with many labs incorporating the program's requirements and standards, with a trained auditor pool prepared to provide accreditation audits and with many labs preparing for the final step of an external accreditation audit.

Contact Information: Karen Snover-Clift, Cornell University, 324 Plant Science Bldg. Ithaca, NY 14853, Phone: 607-255-7860, Email: kls13@cornell.edu

## THE NORTHEAST PLANT DIAGNOSTIC NETWORK (NEPDN); REGIONAL HIGHLIGHTS

**Karen Snover-Clift<sup>1</sup>, Rachel McCarthy<sup>1</sup>, Dawn Dailey O'Brien<sup>1</sup>, Sandra Jensen<sup>1</sup>, Tricia Allen<sup>1</sup>, Emma Rosenthal<sup>1</sup> and Marc Fuchs<sup>2</sup>**

<sup>1</sup>Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY; <sup>2</sup>Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Geneva, NY

The Northeast Plant Diagnostic Network (NEPDN), part of the National Plant Diagnostic Network was established in 2002 and is comprised of 14 diagnostic laboratories from 12 northeastern states. NEPDN membership is committed to communicating information with each other, improving diagnostic skills through professional development workshops and preparing for surge capacity events as they may arise in the northeast and nationally. The NEPDN regional staff and membership have made significant contributions to the national network and to their local agriculture and green industries. NEPDN members have provided leadership in the areas of diagnostics, training and education and the national database, including coordinating the Beltsville significant pest and pathogen workshops, developing and implementing NEPDN's laboratory accreditation program STAR-D; establishing and managing NEPDN's partner program-the Sentinel Plant Network with the American Public Gardens Association; and publishing the monthly NEPDN News. This poster highlights some of the significant activities and contributions made by each of our member states since NEPDN's national meeting in November 2011.

Contact Information: Karen Snover-Clift, Cornell University, 324 Plant Science Bldg. Ithaca, NY 14853, Phone: 607-255-7860, Email: kls13@cornell.edu

## DEVELOPING DURABLE DNA-BASED DIAGNOSTICS: HOW WELL DO YOU KNOW YOUR SOP?

**James P. Stack, Mohammad Arif, Grethel Y. Busot, and Shefali Dobhal**

Department of Plant Pathology, Kansas State University, Manhattan, KS

The development of diagnostic protocols requires more than just identifying a distinguishing feature unique to a given taxon, whether a morphological trait or a DNA sequence. Currently, there are no specific standards established for DNA-based diagnostic protocols for plant pathogens. Such standards should include a clear definition of the diagnostic objectives, the sensitivity and specificity required to minimize false negatives and false positives, the appropriate internal controls for each application, and where the diagnostic should not be used (i.e., the limits of application). Such standards should also provide a framework for protocol validation including guidelines for assembling inclusivity and exclusivity panels based on an understanding of the population genetics and ecology of the target pathogen. Diagnostics work until they fail. Often protocol failure is due to the emergence of new pathogen genotypes or the detection of previously unknown pathogen genotypes. Consequently, the longevity of a protocol may be a function of the validation process. Was the target sequence adequately researched and referenced? Were the inclusivity and exclusivity panels well designed? Were the appropriate internal controls developed and included? Our evolving understanding of microbial populations facilitated by NGS technologies should be considered in the development and validation of plant pathogen diagnostics.

Contact Information: James P. Stack, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, Phone: 785-532-1380, Email: jstack@ksu.edu

**PLANT DISEASE AND PEST DIAGNOSTICS: IT IS MORE THAN YOU THINK**

**C. Tande, F. Mathew, S. Ali, P. Johnson, J. Ball, K. Tilmon, A. Chirumamila, A. Bachmann, O.P. Johnson, B. Fuller, R. Burrows, A. Varenhorst, B. Patrick, and E. Byamukama**

Department of Plant Science, South Dakota State University, Brookings, SD

Plant disease or pest identification is the first step in designing effective and sustainable management programs. The South Dakota State University Plant Diagnostic Clinic helps producers, agronomists, the state department of agriculture, and other stakeholders to diagnose diseases, insects, weeds and nematodes associated with submitted samples. The majority of the samples submitted come from row crops (mainly soybean, corn and wheat) but also from horticultural crops, trees, and turf. The main pathogens diagnosed for plant samples are fungal, followed by bacterial and viral pathogens and a few diagnoses are caused by abiotic factors. Soil samples are usually for the soybean cyst nematode testing but some have been submitted for culturing for root rot pathogens. Submitters of samples receive a diagnosis report and recommendations for management. The state Department of Agriculture uses the clinic services for diagnosis of samples collected in annual surveys. The clinic conducts first responders training throughout the year and participates in various outreach programs in the state. The clinic has produced three first disease reports for South Dakota in the last two years. Occasionally, the clinic also receives unusual and non-plant related samples such as household pests, balls of lint, a dead snake, raccoon feces, moldy eggs, and once, a squishy ball that was “growing alongside the road”. SDSU Plant Diagnostic Clinic is a well-recognized and trusted resource that stakeholders routinely use for a timely and accurate diagnosis and recommendations for management.

Contact Information: Connie Tande, South Dakota State University, SPSB 153, Box 2108, Brookings, SD 57007, Email: [connie.tande@sdstate.edu](mailto:connie.tande@sdstate.edu)

**BUILDING AND EVOLVING PLANT DIAGNOSTICS TO SERVE NEVADA'S AGRICULTURE AND URBAN PLANT HEALTH**

**Shouhua Wang and Rachel A. Bomberger**

Nevada Department of Agriculture, Sparks, NV

Agriculture is an essential part of the rural economy in Nevada. The value of crop production exceeded \$280 million in 2014. Nevada also has diverse ornamental plants and landscape trees, which are valuable assets to 2.7 million urban residents. The Nevada Department of Agriculture Plant Pathology Lab (NDA-PPL) was built to provide plant diagnostic services to state-wide clientele and regulatory programs. As the only plant pathology facility in the state, NDA-PPL has evolved to embrace regulatory, extension, and applied research components to meet the needs of the state. Regulatory plant samples from state inspection and certification programs are diagnosed to support disease regulation, commodity promotion, and export. USDA-PPQ CAPS and Farm Bill survey projects are also conducted to document the absence or presence of exotic pathogens. Samples from general public and extension agents are processed to identify the cause of problems. Diagnostic results and management recommendations help clients to keep plants healthy. To respond to disease outbreaks, NDA-PPL performs research to pinpoint the cause and nature of an outbreak. A rapid response is provided to growers so that the damage to crops can be mitigated effectively. To enhance surge capacity for regulatory disease testing, NDA-PPL introduced USDA-CPHST diagnostic protocols and was certified by the National Plant Protection Laboratory Accreditation Program to perform molecular testing for *Phytophthora ramorum* and citrus greening disease. NDA-PPL adopted the National Plant Diagnostic Network (NPDN) quality management system to ensure a timely, accurate, and reliable diagnosis, and earned the NPDN STAR-D accreditation.

Contact Information: Shouhua Wang, Nevada Department of Agriculture, 405 S. 21st Street, Sparks, NV 89431, Phone: 775-353-3765, Email: [shwang@agri.nv.gov](mailto:shwang@agri.nv.gov)

## TWO SPECIES OF *PHYTOPHTHORA* AND OTHER ROOT PATHOGENS ISOLATED FROM LAVENDER PLANTS FROM ELEVEN STATES IN 2015

Margaret R. Williamson<sup>1</sup>, Suzette R. Sharpe<sup>2</sup>, and Steven N. Jeffers<sup>2</sup>

<sup>1</sup>Department of Plant Industry, Clemson University, Pendleton, SC; <sup>2</sup>Dept. of Agricultural and Environmental Sciences, Clemson University, Clemson, SC

*Phytophthora nicotianae*, a cosmopolitan pathogen with a wide host range, is the most common species of *Phytophthora* attacking herbaceous ornamental and certain woody plants in the southeastern U.S. In June 2015, *P. nicotianae* was isolated from lavender plants (several cultivars of *Lavandula angustifolia* and *L. xintermedia*) with symptoms of *Phytophthora* root and crown rots (PRCRs) that were planted recently at a farm in Greer, SC. Discussions between the SC grower and others in the US Lavender Growers Association (USLGA) revealed that many growers were having similar problems with symptoms of PRCRs—particularly on recently-planted, nursery-grown plants. Growers were encouraged to get plants tested for PRCRs, and the Clemson University Plant Problem Clinic offered to process samples from USLGA members. Between July and November 2015, 29 plant samples were received from 11 states. All plants were cultivars of *L. angustifolia* or *L. xintermedia*. PRCRs were diagnosed on 24 plants based on results of direct isolation from root pieces placed on PARPH-V8 selective medium and crown pieces placed on PARP-V8. *P. nicotianae* was isolated from 19 plants, *P. palmivora* was isolated from four plants, and a species not yet identified was isolated from one plant. Representative isolates from all plants have been stored so additional research can be conducted. In three plants, *Fusarium* crown rot was diagnosed in addition to PRCRs, but the *Fusarium* species most likely were secondary. *Thielaviopsis basicola* was detected as the sole root rot pathogen in two plants, and no root rot pathogens were found in three plants.

Contact Information: Meg Williamson, Clemson University Plant Problem Clinic, Department of Plant Industry, 511 Westinghouse Rd., Pendleton, SC 29670-8841, Phone: 864-646-2133, Email: mwillmsn@clemson.edu

## PARTNERSHIPS



NPDN's collaborative project with the American Public Gardens Association leverages public garden professionals, volunteers and visitors in the early detection of high-consequence pests.

Dr. Shouhua Wang and workshop participants at the Sentinel Plant Network southwestern workshop at Springs Preserve in Las Vegas. photo © Rachel McCarthy, Cornell University

## SEEDY BUSINESS: BIOENERGY AS A NEW SOURCE OF WEEDS

**Jacob Barney**

Virginia Tech, Blacksburg, VA

As with many other regions of the world, the United States is supporting growth of renewable energy, with bioenergy — biomass based energy — being a major component. Many of the species targeted as dedicated bioenergy crops are large statured exotic perennial grasses. The agronomic traits that make them ideal bioenergy crops — perennial, fast growing, few pests, require few inputs, and tolerate poor growing conditions — are also shared by many of our most damaging invasive plants. Additionally, bioenergy crops are likely to be grown widely across the United States integrated among diverse land use types, which may increase the probability of reaching a favorable growing environment. Thus, the combination of traits, large-scale cultivation, and diversity of land use types that may experience propagule pressure makes bioenergy crops unique among agricultural crops in their risk for escape. However, a careful balance of risk mitigation and widespread adoption must be struck as a precautionary approach may unnecessarily stifle an emerging and important industry. Many have proposed a nested-sieve risk assessment approach that combines weed risk assessments, niche and mechanistic modeling, and field studies to characterize and mitigate invasion risk. I will review risk assessment approaches, the current state of knowledge on bioenergy crop invasiveness, and discuss future pest scenarios.

Contact Information: Jacob Barney, Virginia Tech, 435 Old Glade Rd (0330), Blacksburg, VA 24061, Phone: 540-231-6323, Email: jnbarney@vt.edu

## THOUSAND CANKERS DISEASE

**Richard M. Bostock<sup>1</sup>**, Steven J. Seybold<sup>2</sup>, Mohammad Yaghmour<sup>1,3</sup>, Stacy Hishinuma<sup>1</sup>, Tatiana Roubtsova<sup>1</sup>, Tivonne Nguyen<sup>1</sup>, Jason Simmons<sup>1</sup>, Mary Lou Flint<sup>1</sup>, Janine Hasey<sup>3</sup>, Elizabeth Fichtner<sup>3</sup>, Richard W. Hoenisch<sup>1</sup>

<sup>1</sup>University of California, Davis, CA; <sup>2</sup>USDA Forest Service, Davis, CA; <sup>3</sup>UCCE ANR, University of California

Thousand cankers disease (TCD) is an emerging disease first discovered as responsible for dieback and death of eastern black walnut, *Juglans nigra*, in several western states, and now a threat to English walnut, *Juglans regia*, in California orchards. The disease is caused by a fungal infection following attack by the walnut twig beetle (WTB), *Pityophthorus juglandis*, which aggregates and transmits the pathogen, *Geosmithia morbida*. The multiple infections result in numerous, coalescing cankers that girdle and kill branches and stems. TCD is widespread in California and occurs throughout major walnut production areas as well as in woodlands and ornamental stands where native *Juglans* species occur. Our team has characterized the genetic diversity of pathogen and insect vector populations, identified periods of peak beetle activity and host susceptibility, identified an aggregation pheromone for commercial use in monitoring WTB populations, and screened *Juglans* germplasm important for rootstock development against *G. morbida* and attraction for WTB. We have identified natural infections of TCD frequently in black walnut seed trees for rootstock production and in commercial orchards in both English scions and Paradox rootstocks. Our observations suggest interaction between TCD and other diseases and additional, as yet unidentified, factors contributing to tree decline that may provide stresses to facilitate host location or colonization success by the WTB. Of great concern is the vulnerability of the predominant Paradox rootstock in orchards, which is highly attractive to the WTB and most susceptible to the pathogen. There is urgent need to identify methods to protect orchards.

Contact Information: Richard M. Bostock, University of California, Department of Plant Pathology, One Shields Ave., Davis, CA 95616-5712, Phone: 530-752-0308, Email: rmbostock@ucdavis.edu

## SPREADING THE HEAT: INSECT TRANSMITTED PANDEMIC-ASSOCIATED PATHOGENS CONCURRENT WITH CHANGING CLIMATE

**Judith K. Brown**

University of Arizona, Tucson AZ

Recent increases in the volume of international plant trade, expansion of monoculture agriculture, and/or changes in climatic factors has coincided with recent outbreaks caused by a number of emergent and exotic insect-transmitted plant viruses and fastidious eubacterial pathogens. This has resulted in damaging diseases of cacao citrus, cotton, and vegetables, all non-agronomic crops of socio-economic importance in tropical or other mild climate world regions. In particular, investigations point to local upsurges in, or long-distance dispersal of hemipteran insects that specialize in feeding in, and the transmission of plant pathogens that infect, the plant vasculature. The specific relationships between the causal agents and their vectors strikingly implicate altered/increased competency of mealybug-, psyllid-, and whitefly-mediated transmission, potentially aided by endosymbionts obligately-associated with the particular vector haplotype/strain/or species, concomitant with evidence of genomic and geographic expansion of the pathogen, and in some instances, the insect vector as well. Highlighted case studies will explore the pathogen-vector dynamics together with the changing environments that may contribute uniquely and/or universally to the dynamics of the recent outbreaks (*ca.* since 2000) caused by the *Cacao swollen shoot badnavirus*-mealybug complex in West Africa, the citrus and potato/tomato psyllid vector of '*Candidatus Liberibacter*' that infect citrus, or carrot, potato, and tomato crops, respectively, in the Americas and Europe, and the *Cotton leaf curl virus* complex transmitted by the cotton whitefly *Bemisia tabaci* (Genn.) sibling species group in Asia.

Contact Information: Judith K. Brown, School of Plant Sciences, 1140 E. South Campus Dr., University of Arizona, Tucson, AZ 85721, Phone: 520-490-2109, Email: [jbrown@ag.arizona.edu](mailto:jbrown@ag.arizona.edu)

## DIAGNOSTICS IN THE INFORMATION AGE

**Mary Burrows<sup>1</sup> and Carla Thomas<sup>2</sup>**

<sup>1</sup>Montana State University, Bozeman, MT; <sup>2</sup>University of California-Davis, Davis, CA

The use of technology for diagnostics is both a blessing and a curse. Digital diagnostics enables citizens to identify pests of concern in the landscape quickly, cheaply, and accurately. Combined with GPS-located information, it can allow scientists to map pests, create degree day models for prediction, and raise awareness of pests in our ecosystems, engaging citizen scientists in identification and management efforts. However, it can also lead to misdiagnosis, mis-application of pesticides, and overburden scarce subject expert resources. Technology is not going to become less important in the future, so how can we as diagnosticians use the resources available to enhance our impact?

Contact Information: Mary Burrows, Montana State University, 119 Plant BioScience Building, Bozeman, MT 59717-3150, Phone: 406-994-7766, Email: [mburrows@montana.edu](mailto:mburrows@montana.edu)

## INTERNATIONAL DIAGNOSTIC CAPACITY BUILT ON NPDN CAPACITY

**Tom Creswell<sup>1</sup> and Carrie Harmon<sup>2</sup>**

<sup>1</sup>Purdue University, West Lafayette, IN; <sup>2</sup>University of Florida, Gainesville, FL

Accurate and timely disease diagnosis is a cornerstone of a secure food supply, but the capacity for diagnosis is restricted by economics and education, especially in developing countries. The diagnostic trainings and procedures developed and disseminated by NPDN have been propagated into international capacity-building. This presentation will highlight several international programs in the hope of encouraging collaboration and communication among those involved in building diagnostics capacity on an international level.

Contact Information: Tom Creswell, Purdue University, 915 W. State Street, West Lafayette, IN 47907, Phone: 765-494-8081, Email: [creswell@purdue.edu](mailto:creswell@purdue.edu)

**PHYTOPHTHORA RAMORUM DETECTION AND MITIGATION IN EAST COAST RETAIL NURSERIES****Norm Dart**

Virginia Department of Agriculture and Consumer Services, Richmond, VA

In the wake of past shipments of plants infested with *P. ramorum* to east coast nurseries, surveys conducted by eastern states have found few foliar finds of this disease on imported or locally grown nursery stock over the past few years. Using Virginia as a case study; VDACS adapted their *P. ramorum* inspection programs to survey retail and production sites using soil and water baiting. VDACS continues to sporadically find the pathogen in soil and puddling water at retail locations that have received west coast nursery stock but not on plant foliage. These soil and water finds are mitigated by destroying plant material associated with infested soil and water and implementation of best management practices working closely with USDA and other NPDN collaborators. This work is an example of another opportunity for NPDN to continue and to expand current collaborate work with state regulatory organizations.

Contact Information: Norm Dart, Virginia Department of Agriculture and Consumer Services, 600 N. 5th Street, Richmond, VA 23219, Phone: 804-371-5086, Email: norman.dart@vdacs.virginia.gov

**CELL WALL-LESS BACTERIA, PHYTOPLASMAS AND SPIROPLASMAS: CHALLENGES IN DISEASE DIAGNOSIS****Robert E. Davis**

Molecular Plant Pathology Laboratory, USDA Agricultural Research Service, Beltsville, MD

Plant pathogenic phytoplasmas (formerly known as mycoplasmalike organisms, MLOs) and spiroplasmas are minute bacteria whose genomes have become markedly reduced in their adaptations to parasitic life styles alternating between insect vector and plant host. Although they are classified in class *Mollicutes* along with *Mycoplasma* and other wall-less genera, the differing evolutionary trajectories of phytoplasmas and spiroplasmas gave rise to distinctive biological features. For example, phytoplasmas are nonhelical; all phytoplasmas have the same habitat—plant phloem and phloem-feeding insects; and phytoplasmas cannot be isolated in artificial culture. By contrast, spiroplasma cells are helical and motile; spiroplasmas can be isolated in culture; and different *Spiroplasma* species occur as plant pathogens, insect symbionts, and pathogens of crustaceans. Some spiroplasmas even occur on plant surfaces, and spiroplasmas have been implicated in diseases of humans. Although similar technologies are employed for the detection of both phytoplasmas and spiroplasmas, each presents special challenges for disease diagnosis. Among the challenges are complexities due to unresolved taxonomic issues.

Contact Information: Robert E. Davis, Molecular Plant Pathology Laboratory, USDA Agricultural Research Service, Beltsville, MD 20705, Email: robert.davis@ars.usda.gov

**NEWER SPECIES OF ROOT-KNOT NEMATODES****Donald W. Dickson<sup>1</sup> and Janete A. Brito<sup>2</sup>**

<sup>1</sup>University of Florida, Gainesville, FL; <sup>2</sup>Florida Department of Agriculture and Consumers Services, Gainesville, FL

There are some 100+ species of root-knot nematodes identified across the world, and the list keeps growing as new species are discovered. Many of these species are host specific and have limited host ranges whereas other have broad host ranges and are known as soilborne pathogens of great importance to major agricultural crops worldwide. These species include *Meloidogyne incognita*, *M. arenaria*, *M. javanica*, *M. hapla*, and *M. chitwoodi*. During the past 25 years several new species of root-knot nematodes have been discovered. Two of these new species were identified in Florida—*Meloidogyne enterlobii*, in 2002 and more recently *M. floridensis* in 2004. Both of these species are likely to play a major role in Florida agricultural as well that within other states it they get spread about. *M. enterlobii* has been identified in 25 of 65 counties in Florida, and most recently it has been reported in agricultural fields in North Carolina. Currently the presence of *M. floridensis* is limited to Florida but over time we can expect it to infest agricultural fields in neighboring states. The importance of these two species can best be understood when one realizes that they break plant resistant genes in many important agricultural crops that have been bred to be

resistant to the major species of root-knot nematodes. *M. minor*, *M. graminicola*, and *M. hispanica* also have recently been identified infesting agricultural fields in the United States. Also, recently *M. partityla* has been documented to infect other plant species than originally proposed.

Contact Information: Donald W. Dickson, University of Florida, Gainesville, FL, Email: [dwd@ufl.edu](mailto:dwd@ufl.edu)

## SCANNING ELECTRON MICROSCOPY AND MORPHOLOGICAL-MOLECULAR TECHNIQUES

### **Jonathan D. Eisenback**

Virginia Tech, Blacksburg, VA

Imaging the morphology of an organism is critical for the diagnosis of plant-parasitic nematodes and other plant disease agents. Both the light microscope (LM) and the scanning electron microscope (SEM) are extremely useful for studying the structure and form of these organisms. Often observations in one instrument enhance the interpretations of images seen in the other.

The SEM has a very large depth of focus and high resolution, but is limited to imaging the surface of the specimen, whereas the LM has a very narrow depth of focus and lower resolution, but can reveal the internal structures as well. In the recent past molecular techniques have, likewise, become valuable tools for species identification. These electrophoretic patterns of proteins and the DNA or RNA sequences of particular regions of the genome are also morphological structures. The usefulness of these different methods of observation of morphology is directly related to the successful preparation of specimens. Several beneficial techniques in the preparation of specimens for observation in the LM, SEM, electrophoretic gel box, and genomic sequencer will be demonstrated, evaluated, compared, and discussed.

Contact Information: Jon Eisenback, Dept. PPWS, Virginia Tech, Blacksburg, VA 24061, Phone: 540-231-4650, Email: [jon@vt.edu](mailto:jon@vt.edu)

## MORPHO-MOLECULAR TAXONOMY TOOLS FOR INSECTS

### **Raymond Gill**

California Department of Food & Agriculture, Sacramento, CA

Benefits and problems associated with morphological taxonomy and species identifications in conjunction with molecular techniques are discussed. These pros and cons relating to this topic are based on many personal experiences gained over many years working in the field of insect taxonomy. Reasons are discussed as to why morphological study and classical taxonomy are very often inadequate or incapable of reaching a conclusion as to the exact identification of a given specimen or population. Helpful results have been obtained by combining classical methods with molecular data using such tools as electrophoresis and DNA based techniques. Barcoding of specimens is discussed, along with issues about the appropriateness of this kind of tool as an actual aid to the daily work of the taxonomist and port identifiers. And it was suggested that a very important use of molecular taxonomic work would be the ability to identify all life stages of a specimen. Examples of mealybug and whitefly species are used to suggest some of the benefits of using both techniques together and to suggest strongly that the two techniques must be used together and that they cannot be mutually exclusive.

Contact Information: Raymond Gill, California Department of Food & Agriculture, 3294 Meadowview Rd., Sacramento, CA, Phone: 916-525-2780, Email: [rgill@cdfa.ca.gov](mailto:rgill@cdfa.ca.gov)

## NPDN'S POSITIVE IMPACTS ON DIAGNOSTICS AND EXTENSION PROGRAMMING

### **Ray Hammerschmidt**

Michigan State University, East Lansing, MI

One of the missions of the Land Grant Universities is to provide research-based information to the citizens and stakeholders of their state that can assist them in improving their lives. This information is delivered through the state-wide networks that make up Cooperative Extension. An important part of this effort are the diagnoses and

related plant disease and pest information that is provided through the efforts of the plant diagnostic laboratories. Since 2002, the NPDN has had numerous accomplishments that have positively impacted the diagnostic community and have enhanced the ability to provide accurate and timely diagnoses. Notable are the improvements in laboratory infrastructure, educational materials, and communication within the diagnostic community. In-depth training opportunities for diagnosticians have provided cutting edge diagnostic protocols for the labs, and a variety of exercises with the labs, state departments of agriculture, USDA and stakeholders have built strategic relationships. In total, the accomplishments of the NPDN have provided needed resources to support 21st Century plant diagnostics, and this support has allowed the diagnostic labs to strengthen Extension efforts and build stronger relationships with state, federal and private sector partners.

Contact Information: Ray Hammerschmidt, Michigan State University, East Lansing, MI, Email: hammersr@anr.msu.edu

## RAPID DETECTION OF CITRUS BLACK SPOT WITH LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

Kara Levin<sup>1</sup>, Kurt Zeller<sup>1</sup>, Gang Wei<sup>1</sup>, Gloria Abad<sup>1</sup>, Zhaowei Liu<sup>1</sup>, Hilda Gomez<sup>2</sup>, Timothy Riley<sup>4</sup>, Geoffrey Dennis<sup>3</sup>, Charla Hollingsworth<sup>3</sup>, Mark Nahkla<sup>1</sup>

<sup>1</sup>USDA PPQ: Beltsville, MD; <sup>2</sup>USDA PPQ: Davie, FL; <sup>3</sup>USDA PPQ: Raleigh, NC; <sup>4</sup>USDA PPQ: Orlando, FL

Citrus Black Spot (CBS) caused by *Guignardia citricarpa* (*Phyllosticta citrocarpa*, anamorph) is a serious fungal disease of citrus crops, worldwide. The pathogen causes unsightly lesions that render fresh fruits unmarketable. CBS was first detected in Southern Florida in 2010 and its presence has since been confirmed in three Florida counties. Currently, packing facilities in Florida inspect for CBS diseased fruit from September to April for export shipments to the EU. If suspect symptomatic fruit is intercepted during inspection, the entire shipment of this perishable commodity is delayed at packing houses. Operation managers need rapid diagnostics to assess individual shipments in a timely manner in order to facilitate business. Microscopic examination of symptomatic fruit is not sufficient to provide confirmation for shipment release. Current systems in place for confirmatory diagnostics of *G. citricarpa* must be performed at off-site laboratories and include a 60–90 minute Qiagen extraction method followed by a 75 minute real-time program, all of which impose a significant delay in result reporting. In this work, we assessed a Loop-Mediated isothermal **AMPlification** (LAMP) technology to develop an easy and rapid detection system. With the use of LiNK technology, the extraction time was reduced to 12 minutes. Our preliminary results indicate that the LAMP assay is highly sensitive and specific comparable to real-time PCR. It is also able to replicate quantitative real-time PCR diagnostics of environmental samples in 8–21 minutes in a non-laboratory environment. This LAMP assay offers a total test time including sample processing of less than 45 minutes.

Contact Information: Kara Levin, USDA-APHIS-CPHST, BARC-East, Bldg. 580, Powder Mill Rd., Beltsville, MD, USA 20705, Phone: 301-313-9244, Email: kara.levin@aphis.usda.gov

## ON SITE RAPID ISOTHERMAL DETECTION OF PHYTOPHTHORA SPECIES

**Frank Martin**

USDA-ARS, Salinas, CA

Comparative analysis of mitochondrial genomes has been used to develop a sensitive and specific assay for detection of *Phytophthora*. To improve specificity, conserved primers were designed to amplify unique gene orders for this genus when compared to related genera, Eumycotan fungi and plants. The assay was designed for the genus specific amplicon to have annealing sites for a genus and species specific TaqMan probes. Thus far species specific probes have been validated for 50 taxa with sequence data indicating markers should be able to be developed for approximately 85% for the genus.

The TaqMan assay was modified for use with the isothermal technology recombinant polymerase amplification (RPA) by using the TaqMan genus specific probe annealing site for design of the RPA probe and the species specific TaqMan probe sequence for design of the RPA species specific reverse primer. A genus specific and 9 species specific assays have been validated (including *P. kernoviae* and *P. ramorum*); using this approach provides a systematic approach for development of additional species specific assays. When used with a portable fluorimeter

these assays provide in-field detection capabilities that can be completed in as little as 15 minutes without the need for DNA extraction and a sensitivity approaching TaqMan real time PCR. To confirm species identification when a positive is obtained, techniques were developed for PCR amplification of the *Phytophthora* genus specific amplicon directly from the RPA amplification; subsequent sequence analysis and querying our database of over 900 isolates representing 136 *Phytophthora* taxa confirms species identification.

Contact Information: Frank Martin, USDA ARS, 1636 East Alisal St., Salinas, CA 93905, Phone: 831-755-2873, Email: frank.martin@ars.usda.gov

## COMBINING FORCES TO DETECT INVASIVE FOREST PESTS

### Deborah G. McCullough

Department of Entomology and Department of Forestry, Michigan State University

Invasive insects in forests and urban forests are economically costly, affect a wide range of ecological services, and disrupt numerous ecosystem processes. Early detection of newly established populations of invasive forest insects is a key element of successful eradication or management of these pests. Early detection is especially difficult, however, when little is known about a non-native species or when highly effective lures and traps are not available. Reports from citizen scientists can supplement regulatory or research surveys, but only if a mechanism is in place to evaluate and respond to these reports. In Michigan, for example, the Eyes on the Forest project was recently initiated to expand awareness and increase the likelihood that newly established invasive forest pests will be detected early. One aspect of the project involves recruiting volunteers with relevant expertise who agree to adopt a sentinel tree(s), periodically monitor the tree's condition and submit their observations through the Sentinel Tree Network website. Observations of unusual tree decline or suspicious symptoms can be assessed by regulatory or forest health professionals but the negative reports—i.e., the distribution of healthy trees—also provide valuable information. Specialists from the MSU Diagnostic Services group support this and related efforts by quickly evaluating suspect images or insect specimens and communicating results to the observer and, when required, entering the specimen into the appropriate regulatory pathway.

Contact Information: Deborah McCullough, 288 Farm Lane, 243 Natural Science Building, East Lansing, MI 48824; Phone 517-355-7445; Email: mccullo6@msu.edu

## THE ROLE OF NPDN IN DIAGNOSTIC CAPACITY DURING DOMESTIC OUTBREAKS: PHYTOPHTHORA RAMORUM, A WEST COAST PERSPECTIVE

### Nancy K. Osterbauer

Oregon Department of Agriculture, Salem, OR

*Phytophthora ramorum*, the cause of sudden oak death and related diseases, was first reported infecting and killing tanoaks in California in 2002. Subsequently, the pathogen was detected in large interstate shipping nurseries on the West Coast in 2004; these nurseries had unknowingly shipped infected plants to 176 different sites in 22 states. This outbreak prompted the adoption of an interim federal quarantine designed to prevent further spread of the pathogen via plants for planting and demonstrated the need for the National Plant Diagnostic Network or similar entity as a means to assist with rapid, reliable, and accurate detection of the pathogen. The situation on the West Coast differed from the situation on the East. The State Departments of Agriculture in California, Oregon, and Washington, the states most immediately affected by the federal quarantine, used federal funding to upgrade their laboratory facilities to meet the surge in testing capacity. Other, university-based Western Plant Diagnostic Network laboratories also benefited from an influx of federal funds to upgrade their facilities to assist with testing. However, the majority of testing remained within the State Departments of Agriculture. As the federal regulations for *P. ramorum* have changed over time, the need for university-based WPDN laboratories to provide official *P. ramorum* testing has waned, although their ability to serve as First Responders and points of contact for the general public remains critical as demonstrated by recent *P. ramorum* detections in urban environments in Oregon and Washington.

Contact Information: Nancy K. Osterbauer, Oregon Department of Agriculture, 635 Capitol St NE, Salem, OR 97301, Phone: 503-986-4620, Email: nosterbauer@oda.state.or.us.

**THE IMPACT OF SYSTEMATICS ON THE IDENTIFICATION OF FUNGI****Megan K. Romberg**

USDA APHIS, Washington, D.C.

Accurate identification of the fungi associated with a given set of symptoms has consequences for disease management and regulatory decisions. It also provides information on the distribution and spread of a particular fungus. Identification of fungi relies on comparison of observed characters to those used to describe a given species, which in turn relies on systematics to clarify the relationship of taxa within genera and at higher taxonomic levels. Estimates place the number of fungal species in the world at between 1.5 and 6 million, of which approximately 100,000 have been described. Modern systematic research has covered only a fraction of the described fungi. Hence, development of specific diagnostic tests for certain fungi is hampered by the lack of a solid framework covering a wide variety of representatives in a group. Systematic research also impacts the names given to fungi, including synonymizing different states as per recent changes under the one name for fungi rules of the International Code of Nomenclature. Recent examples demonstrating the need for, or utility of, broad systematic research will be provided. Additionally, ideas for ways in which NPDP members can contribute to global systematic research on fungi will be discussed.

Contact Information: Megan Romberg, USDA APHIS, 10300 Baltimore Ave., Beltsville, MD 20705, Phone: 301-313-9391, Email: [megan.k.romberg@aphis.usda.gov](mailto:megan.k.romberg@aphis.usda.gov)

**AN OVERVIEW OF ISOTHERMAL AMPLIFICATION PLATFORMS IN GENERAL AND AMPLIFYRP® SPECIFICALLY****Paul F. Russell, Nathan McOwen, Bryant Davenport, Shulu Zhang, and Rugang Li**

Agdia, Inc., Elkhart, IN

Molecular detection of pathogens has become a mainstay for plant diagnostics, PCR-based methods being the go-to platform. However, PCR-based methods mostly rely on the use of purified DNA or RNA as template and require very costly laboratory equipment, thus increasing cost and labor. Isothermal amplification platforms have more recently gained favor because of tolerance to host inhibitors and their use of simple instrumentation.

The list of isothermal platforms is a long one which includes helicase-dependent amplification, loop-mediated amplification, rolling circle amplification, recombinase polymerase amplification and others. Agdia's AmplifyRP® platform is an isothermal amplification system based on the recombinase polymerase amplification system. Detection of amplification can be performed in real-time using a fluorometer (XRT) similarly to realtime PCR, or as an end-point assay using lateral flow strips in an Amplicon Detection Chamber (Acceler8). Both formats are extremely sensitive and specific as qualitative assays.

Contact Information: Paul Russell, Agdia, Inc., Research & Development, 52642 County Road 1, Elkhart, IN 46514, Phone: 574-264-2014, Email: [prussell@agdia.com](mailto:prussell@agdia.com)

**EDNA METHODOLOGY: E-PROBE DIAGNOSTIC NUCLEIC ACID ANALYSIS****William Schneider**

USDA ARS, Fort Detrick, MD

Plant pathogen detection takes many forms. In simple cases, researchers are attempting to detect a known pathogen from a known host utilizing targeted nucleic acid or antigenic assays. However, in more complex scenarios researchers may not know the identity of a pathogen, or they may need to screen for a wide array of pathogens from a single sample. Metagenomics represents a possible broad range diagnostic approach, with next generation sequencing generating a comprehensive profile of all organisms in a given nucleic acid sample. However, the vast amount of data gathered makes metagenomic analysis computationally problematic. EDNA is a bioinformatic tool that greatly reduces the amount of computational work in metagenomic data diagnoses. EDNA reverses the process of metagenome analysis. Instead of comparing the entirety of a large dataset to a large and flawed reference database, EDNA treats the metagenome as a database that is searched using key diagnostic sequences called

e-probes. This allows for complete and accurate diagnostic analysis of metagenomes in minutes using a standard laptop computer. EDNA has been utilized to detect RNA and DNA viruses, bacteria, fungi, oomycetes, human pathogens on plants and insect vectors. The cost of metagenome sequencing is currently high, but the ability to do unlimited multiplex identification of unlimited numbers of pathogens and/or vectors in a single assay means that EDNA is economically feasible in situations where individual assays require many tests, such as in quarantine facilities.

Contact Information: William Schneider, USDA ARS, Fort Detrick, MD, Email: [william.schneider@ars.usda.gov](mailto:william.schneider@ars.usda.gov)

## TALES OF TWO HARDWOOD BORERS: POLYPHAGOUS SHOT HOLE BORER AND GOLDSPOTTED OAK BORER

**Steven J. Seybold<sup>1</sup>, Yigen Chen<sup>2</sup>, Tom W. Coleman<sup>3</sup>, Lori J. Nelson<sup>1</sup>, Robert C. Venette<sup>4</sup>**

<sup>1</sup>USDA Forest Service, Davis, CA; <sup>2</sup>University of California, Davis, CA; <sup>3</sup>USDA Forest Service, San Bernardino, CA; <sup>4</sup>USDA Forest Service, St. Paul, MN

Two emerging invasive pests of hardwood trees in southern California present an interesting contrast at many levels. The polyphagous shot hole borer is an ambrosia beetle from Southeast Asia that utilizes pathogenic fungi to rapidly colonize and kill numerous hardwood tree species. This pest has spread from an initial detection in 2003 near the Port of Long Beach, and threatens many ornamental and native riparian hardwood trees in the LA Basin, as well as many fruit and nut crop trees of the Coastal and Central Valleys. The goldspotted oak borer is a flatheaded borer from southeastern Arizona that slowly colonizes large diameter oaks primarily in the red oak group (*Quercus*, Section *Lobatae*). This beetle does not rely on a fungal symbiont to kill trees. It has spread comparatively slowly from an initial detection in 2004 in eastern San Diego County, but satellite populations have appeared with increasing frequency in concert with unregulated movement of oak firewood in the State. Both species can be diagnosed from their tree damage characteristics, however, based on insect morphology alone, adult polyphagous shot hole borers are difficult to distinguish from closely related invasive taxa in the USA (e.g., the tea and Kuroshio shot hole borers), whereas adult goldspotted oak borers are easily identified. Diagnostic techniques are available for both species, i.e., DNA sequencing or chemical analysis of cuticular hydrocarbon profiles. Preliminary risk analyses for both species suggest that their ranges will continue to expand in California and perhaps into adjoining states and Mexico. Movement of infested firewood is likely to be the key to the rate of that expansion.

Contact Information: Steven J. Seybold, USDA Forest Service, Pacific Southwest Research Station, 1731 Research Park Drive, Davis, CA 95618, Phone: 530-752-6231, Email: [sjseybold@gmail.com](mailto:sjseybold@gmail.com) or [sseybold@fs.fed.us](mailto:sseybold@fs.fed.us)

## MULTIPLEX FOR TOMATO DISEASES

**Christine Smart**

Cornell University, Geneva NY

Rapid and accurate detection and identification of pathogens is critical for plant disease management. DNA array technology has been successfully applied for simultaneous detection of multiple microorganisms from various habitats. The goal of this project was to develop a multiplex detection/identification system for the major fungal and oomycete pathogens of tomato. To facilitate this goal, we utilized a membrane-based macroarray that included at least two specific oligonucleotides per pathogen. A total of 105 oligonucleotides specific for about 30 pathogens of tomato were designed based on the internal transcribed spacer sequences of the rDNA and spotted on a nylon membrane. The array was tested against each target pathogen species, 46 infected field samples, and a number of non-target species. Our results indicate that the oligonucleotide-based macroarray detection system is a reliable and effective method for pathogen detection and identification even when multiple pathogens are present in a field sample. Because of our success with macroarray detection that was based on a small number of single nucleotide polymorphisms, we are now using high resolutions melt analysis and locked nucleic acid probes to specifically detect and identify pathogens or strains within a pathogen species.

Contact Information: Christine Smart, Cornell University, 630 W. North St, Geneva, NY 14456, Phone: 315-787-2441, Email: [cgs14@cornell.edu](mailto:cds14@cornell.edu)

**HOLY GUACAMOLE: INSIGHTS INTO THE EMERGING LAUREL WILT PANDEMIC**

**Jason A. Smith<sup>1</sup>**, Marc Hughes<sup>1</sup>, Lanette Sobel<sup>1</sup>, Carrie L. Harmon<sup>2</sup>, Tyler Dreaden<sup>1</sup> and Randy C. Ploetz<sup>3</sup>

<sup>1</sup>School of Forest Resources and Conservation, University of Florida: Gainesville, FL; <sup>2</sup>Plant Disease Center, University of Florida: Gainesville, FL; <sup>3</sup>UF-TREC: Homestead, FL

Laurel wilt has been causing significant mortality to trees in the Lauraceae in the southeastern United States since 2002. In little more than a decade, the Asian redbay ambrosia beetle (*Xyleborus glabratus*) and its fungal symbiont *Raffaelea lauricola* have spread to nine states, killing hundreds of millions of redbay trees. The impacts of the epidemic are diverse. Major changes have occurred in ecosystems including changes in species compositions, reductions in populations of associated butterfly species, and increased vulnerability of fragile systems such as that in the Everglades. Additionally, LW is now responsible for substantial losses in the avocado industry (valued at > \$60 million/year in Florida) and of cultural resources for the American Indian community. Typically, ambrosia beetles carry and utilize nonpathogenic fungi as nutritional symbionts. LW is unique in that the symbiont of *X. glabratus* is highly virulent to host trees. Microscopic observations and studies with secondary metabolites indicate that symptomatic hosts produce high numbers of tyloses, which impede vascular function. Genetic analyses indicate that a single clone of *R. lauricola* occurs in the USA, possibly introduced from Taiwan. Diagnosis relies on PCR amplification of two species-specific SSR loci. Current efforts to manage LW focus on systemic fungicides, insect repellents, sanitation and host resistance. Disease tolerant redbay clones have been propagated and are being used in studies aimed at understanding underlying genetic mechanisms of tolerance. Coppice management is being explored as a management practice in natural forests. Future research will focus on genomic analyses of the pathogen as well examinations of host susceptibility within the Lauraceae.

Contact Information: Jason A. Smith, University of Florida, Gainesville, FL, Email: [jasons@ufl.edu](mailto:jasons@ufl.edu)

**TRACKING UG99 AND OTHER RACES OF THE WHEAT STEM RUST PATHOGEN USING SNP TECHNOLOGIES**

**Les J. Szabo<sup>1</sup>**, Jerry L. Johnson<sup>1</sup>, David Hodson<sup>2</sup>, Pablo D. Olivera<sup>3</sup>, Yue Jin<sup>1</sup>

<sup>1</sup>USDA Agricultural Research Service, Saint Paul, MN; <sup>2</sup>CIMMYT-Ethiopia, Addis Ababa, Ethiopia; <sup>3</sup>University of Minnesota, Saint Paul, MN

Stem rust continues to be a threat to wheat production and global food security. A new highly virulent race (Ug99) of the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*, Pgt) was first collected in Uganda in the late 1990's and has caused severe epidemics in northeast Africa. In the last decade ten new race variants of Ug99 have been identified and members of Ug99 race group are widely distributed in eastern Africa and the Middle East. Standard method for determining Pgt race phenotypes requires living cultures and takes several weeks to more than a month. Recent availability of genomic resources for Pgt, has allowed the development of DNA based molecular diagnostic tools for rapid detection and monitoring critical race groups. A primary component of the current international surveillance program for Pgt Ug99 race group uses a two-stage qPCR assay and non-living samples. In 2013 a stem rust epidemic devastated the wheat crop in southern Ethiopia and characterization of Pgt isolates from this epidemic has determined that another lineage of Pgt was responsible. Genotyping of these isolates allowed for the rapid development of new qPCR assay for this Pgt lineage. Pgt has been called the "Shifty Enemy" and it is living up to its name. The dynamic nature of the global Pgt population requires continuing collaboration of the international wheat rust community in order that the diagnostic methods stay current and relevant.

Contact Information: Les J. Szabo, USDA Agricultural Research Service, Saint Paul, MN, Email: [les.szabo@ars.usda.gov](mailto:les.szabo@ars.usda.gov)

## IPM INNOVATION LAB (CRSP) DIAGNOSTICS TRAINING AND ESTABLISHMENT IN DEVELOPING COUNTRIES

**Sue A. Tolin**

Virginia Tech, Blacksburg, VA

The necessity for accurate diagnosis of plant disease agents was recognized early in the 20-plus year history of the USAID-funded Integrated Pest Management - Cooperative Research Support Program, now Innovation Lab. Two global theme projects were funded to assess and provide diagnostic capabilities for viruses and other pathogens, introduce new methodologies, identify constraints, and provide training and resources to build in-country capacity. The infrastructure, resources, and human capacity to access and use modern diagnostic methods varied greatly among collaborating countries, leading to a variety of approaches. This talk will highlight selected activities in diagnostics impacting the design of ecologically-based disease management approaches.

Contact Information: Sue A. Tolin, Virginia Tech, Blacksburg, VA 24060, Phone: 540-231-5800, Email: stolin@vt.edu

## RATHAYIBACTER TOXICUS: A DUAL KINGDOM PATHOGEN THREATENING PLANTS, ANIMALS AND HUMANS

**Anne Vidaver**

University of Nebraska, Lincoln, NE

*Rathayibacter toxicus* causes an animal disease, usually fatal, through ingestion of infected plants that may or may not show yellow gummosis caused by the bacterium, which in turn is brought into the plant seedhead by nematodes carrying the adherent bacteria. Poisonous toxins are produced that cause convulsions, aberrant behavior and often rapid death by grazing animals. Recovery seldom occurs and usually is incomplete. The glycolipid toxin(s) are poor immunogens so that recovery is rarely known. While advances in knowledge of the pathogen are occurring, the genetic basis for pathogenicity and virulence factors, and the numerous toxins (16 known) produced remain unknown. Principally a disease in several areas of Australia, it affects all grazing animals, especially sheep and cattle. Management involves careful monitoring of pastures, especially of annual ryegrass (*Lolium rigidum*), resistant cultivars (some available), herbicides, biological control (varied results), and certified seed free of the bacterium. Complexities associated with nematode vectors, presumptive role of bacteriophages, *R. toxicus* and its toxins, plant susceptibility and grazing animals make this one of the most challenging diseases for plant pathologists and veterinarians.

Contact Information: Anne Vidaver, Dept. Plant Pathology, 406 Plant Science Hall, UNL, Lincoln, NE 68583, Phone: 402-472-2812, Email: avidaver1@unl.edu

## IMPORTANCE OF NEMATODES ON TURFGRASSES AND LANDSCAPE PLANTS

**Fred Warner**

Michigan State University, East Lansing, MI

Plant-parasitic nematodes are frequently recovered from turfgrasses and landscape plants but their impacts are often not well documented or understood, especially the economic losses. This is one of four inconvenient truths covered in this presentation. Although evidence suggests turf managers in many regions of the US often ignore nematodes, it appears, especially in many areas, nematode problems are on the rise on golf course greens. The movement away from most carbamate and organophosphate insecticides/nematicides is given as one reason to explain the trend. Some newer observations regarding nematodes on turf in the North Central US are provided. Damage thresholds for nematodes on turf, especially cool-season species, are not well established. Nurserymen, landscapers and greenhouse operators also tend to ignore plant-parasitic nematodes unless phytosanitary certificates are needed in order to export plants. Numerous plant species and substantially more cultivars of these species are grown in gardens/landscapes and in Michigan, 21 genera of plant-parasitic nematodes have been recovered from these plants. Long distance transport of plant-parasitic nematodes occurs in plant material. In Michigan, the burrowing nematode, *Radopholus similis*, exists in the W.J. Beal Botanical Garden on the campus of MSU although the northern most distribution of this nematode in the US was believed to be Florida. It appears

to successfully overwinter on *Miscanthus* sp., although it was undoubtedly introduced on a banana plant. This represents just one example where an exotic nematode was accidentally introduced to an area. A few statements provided in a paper written by Dr. Axel Elling, while at Washington State University, on minor Meloidogyne species conclude the presentation.

Contact Information: Fred Warner, Diagnostic Services, 578 Wilson Road, Michigan State University, East Lansing, MI 48824-6469, Phone: 517-432-1333, E-mail: fwnemalb@msu.edu, www.pestid.msu.edu

## HOW TO GET YOUR LAB INVOLVED IN TESTING FOR EXPORT OR IMPORT

### Weimin Ye

North Carolina Department of Agriculture & Consumer Services, Raleigh, NC

The Nematode Assay Lab in Agronomic Division of North Carolina Department of Agriculture & Consumer Services (NCDA&CS) is the largest high-throughput and publicly operated nematode assay lab in the USA. We provide nematode diagnosis services to the farmers from North Carolina and officers from the USDA Animal and Plant Health Inspection Service and NCDA&CS Plant Industry in support of the issuance of phytosanitary certificates for export of plant materials. The regulated nematode species requested for testing include the pine wood nematode (PWN, *Bursaphelenchus xylophilus*), soybean cyst nematode (*Heterodera glycines*), reniform nematode (*Rotylenchulus reniformis*), burrowing nematode (*Radopholus similis*), stem and bulb nematode (*Ditylenchus dipsaci*, *D. destructor*) and any cyst nematode species. In the past few years, due to more strict regulations on PWN, a large number of pine-wood samples were submitted to this lab. In fiscal year 2013 and 2014, 17,592 pine-wood samples were analyzed which accounted for 21.3% of our total samples. PWN was detected from 2.1% of these samples. In addition to the traditional morphological identification, we characterized the DNA sequences on the ribosomal DNA small subunit, large subunit D2/D3, internal transcribed spacer (ITS-1) and mitochondrial DNA cytochrome oxidase subunit one on the Aphelenchid species and described the development of a real-time-PCR method for rapid and accurate identification of PWN using a TaqMan assay targeting the ITS-1. This assay proved to be specific to PWN only and was sensitive to a single nematode specimen regardless of life stage.

Contact Information: Weimin Ye, Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture & Consumer Services, Raleigh, NC 27607, Phone: 919-733-2655, Email: weimin.ye@ncagr.gov

Moko disease (*Ralstonia solanacearum*) on plantain in Columbia. photo © Tom Creswell, Purdue University



“The diagnostics workshop in Columbia provided an opportunity for us to see tropical fruit production problems first hand, teach diagnostics techniques, and gave participants a chance to practice applying them to a range of disease samples.”

~Tom Creswell, Purdue University

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*WPDN, University of California, Davis*



### Jacob Barney

Dr. Jacob Barney is an Assistant Professor of Invasive Plant Ecology at Virginia Tech. He received his BS in chemistry from the University of Kentucky where he worked in a weed science lab. Jacob received his MS and PhD from Cornell University in weed ecology. Jacob moved to the University of California Davis as a Postdoctoral Scholar before beginning his position in Blacksburg in 2010. Jacob's research program is focused on broad aspects of weed and invasive plant biology, ecology, and management. Jacob has research programs in a variety of systems, including deciduous forests, reclaimed coal mines, riparian systems, and agroecosystems. Jacob also developed an innovative research program on determining the invasive potential of biofuel crops. Jacob has published 51 peer-reviewed papers in 31 journals, 1 book, 6 book chapters, 3 policy documents, 2 extension publications, and given

25 invited and 83 contributed presentations. Jacob has been awarded \$1.6M in grants and contracts primarily from the USDA. Jacob has been honored to receive the Weed Science Society of America's Early Career Outstanding Researcher Award and the Outstanding Graduate Student Award, and Northeastern Weed Science Society's Outstanding Researcher Award and the Robert D. Sweet Outstanding Graduate Student Award. Jacob also serves as an Associate Editor for *Invasive Plant Science and Management*.

### Richard Bostock

Rick Bostock is a professor in the Department of Plant Pathology at the University of California, Davis, and the Director of the Western Region of the National Plant Diagnostic Network (NPDN). Dr. Bostock received his B.S. degree in biology from Rhodes College in 1974 and a Ph.D. in plant pathology at the University of Kentucky in 1981. He has been a faculty member at UC Davis since 1981. His research and teaching interests are the biochemistry and molecular biology of plant-microbe interactions, which has included a long-standing interest in diseases caused by *Phytophthora* species. Within this general area of research, he and his colleagues have studied lipid-based signaling in plant immunity and the coordination of plant stress signaling networks to enhance or diminish disease resistance. In addition to these basic studies, he leads an active applied research program on fungal diseases of orchard crops. In 2002, he was appointed as the founding Director of the western region of the NPDN, and through his participation in the NPDN, he has become increasingly involved in research and mitigation of new and emerging plant pathogens. Of particular current interest in his lab is thousand cankers disease of walnuts, which is caused by an insect-vectored fungal pathogen. Rick teaches several undergraduate and graduate courses in plant pathology, as well as a popular course entitled "*Feeding the Planet: Challenges to the Global Food Supply*" in the Science and Society program. He was chair of the Department of Plant Pathology from 1999–2005. He is a Fellow of the American Phytopathological Society and a Fellow of the American Association for the Advancement of Science.



### Judith K. Brown

Brown, a professor of plant pathology/plant sciences in the School of Plant Sciences, College of Agriculture and Life Sciences, University of Arizona earned her doctorate from the UA in 1984, M.S. from Washington State University in 1981, and undergraduate degree from Texas A&M University in 1979. Brown has been involved as a UBRP mentor, has studied emerging insect-transmitted viral and bacterial pathogens of plants in more than 65 countries, and has carried out collaborative research in Asia, Africa and the tropical Americas. She is a world authority on whitefly-transmitted and other emerging plant viruses and vector biotypes. Her work uses DNA tools to understand causal agents and ways to control them in disease-stricken crop plants, often on a grand scale. Examples include geminivirus-caused pandemics of cotton in Pakistan, India and China and of cassava crops in sub-Saharan Africa countries.

In the Americas, Brown is researching DNA and RNA whitefly-transmitted viruses affecting vegetables, and psyllid-borne pathogens that cause tomato vein greening and citrus greening. Currently, she is studying a new outbreak of Cacao swollen shoot virus in West Africa. Brown shares her findings, which can affect



local economies and food supplies, through numerous task forces, working groups, consultancies and presentations globally. Her laboratory has hosted more than 60 visiting scholars and graduate students from more than 30 countries. For her contributions, Brown was awarded the Washington State Women's History Recognition for Professional & Academic Leadership in 2005, the Wellman Award- Professional Achievement and Leadership by the APS-Caribbean Division in 2008, named a Fellow by the American Phytopathological Society in 2011, and by the American Association for the Advancement of Science in 2015.

### **Mary Burrows**

Dr. Mary Burrows obtained her PhD in Plant Pathology from the University of Wisconsin-Madison and did a post-doc at the USDA-ARS at Cornell University. She started her position as the Extension Plant Pathology Specialist at Montana State University in August of 2006. Her extension and research activities focus on crops including wheat and pulses. She also directs the Schutter Plant Diagnostic Laboratory, the Pulse Crop Diagnostic Laboratory, serves as the Integrated Pest Management coordinator for Montana, and has an active applied research program.



### **Tom Creswell**

Tom Creswell has been with Purdue University for eight years, serving as director of the Plant and Pest Diagnostic Laboratory. His expertise is in the area of diagnostics of ornamental plants, vegetables, fruit and specialty crops. He has supported Purdue's international extension work by providing introductory plant pathology training to plant protection faculty from eight Afghanistan universities. He has also trained specialists in plant disease diagnostic methods and laboratory management standards in workshops in Afghanistan, Bangladesh, Grenada and Columbia. His recent collaborations include working with 10 other diagnostic labs to develop a wide range of online diagnostic resource materials for use by extension personnel and diagnosticians and development of a sample submission app for plant diagnostic labs.

Prior to Purdue University, Tom was the managing director of the Plant Disease and Insect Clinic at North Carolina State University (NCSU) for 20 years.

He received his B.S. in Botany (1980) and M.S. in Plant Pathology (1982) from Auburn University and his Ph.D. in Plant Pathology (1987) from NCSU.

### **Norm Dart**

Norm Dart has a MS in plant pathology and is the State Plant Pathologist for Virginia. He has worked in the field of regulatory and research plant pathology for 12 years specializing in soil borne pathogens of nurseries. Norm also has passion and expertise in forest pathology and ecology. He works closely with nursery, landscape professionals, USDA, consumers and Universities to serve the interests of all stakeholders.

### **Robert E. Davis**

Robert E. Davis, Ph.D., is Research Leader of the USDA-ARS Molecular Plant Pathology Laboratory (MPPL) at the Beltsville Agricultural Research Center. His personal research focuses on diseases that once were believed to be caused by viruses, but now are known to be caused by cell wall-less bacteria called phytoplasmas and spiroplasmas. Dr. Davis is the discoverer of spiroplasmas, and he coined the term spiroplasma. Spiroplasmas are now known to infect diverse insects and ticks, to cause diseases in crustaceans, and in 2015 a spiroplasma was reported as the cause of a systemic infection in a human. As Research Leader of the MPPL, Dr. Davis oversees broad and in-depth research on cellular plant pathogens (walled bacteria, phytoplasmas, spiroplasmas) and subcellular plant pathogens (viruses and viroids). The research is aimed at understanding the molecular biology of plant disease agents and pathogen-host interactions. The findings provide fundamental, new scientific knowledge that is



used to construct new concepts and devise new tools for detection and identification of pathogens, modification of plant viruses for production of useful products including vaccines and nano particles in plants, and the development of new and environmentally safe disease control measures. Dr. Davis holds a B.Sc. degree from the University of Rhode Island and a Ph.D. from Cornell University. In recognition of his research accomplishments, Dr. Davis has received numerous prestigious awards and is a member of the USA National Academy of Sciences.

### Donald Dickson

Professor Dickson has worked as a researcher, extension specialist, and teacher of nematology for over 50 years, 47 of which have been spent at UF. He is considered as an authority of nematode management on horticultural and agronomic crops; biological control of plant-pathogenic nematodes, and the ecology and distribution of root-knot nematodes. He is knowledgeable about soil fumigant and nonfumigant applications in both vegetable and row crop production.



### Jonathan Eisenback

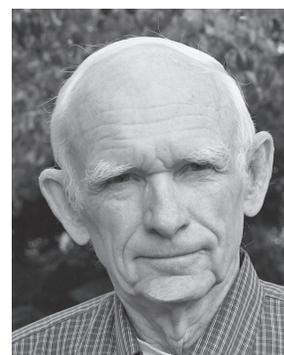
Jon Eisenback received a B.A. in Biology from Bryan College and taught ninth grade science in Rhea County Consolidated High School. He received a Ph.D. in plant pathology from North Carolina State University for his work on the morphology and taxonomy of the root-knot nematodes. Jon was a post-doc for several years in the International *Meloidogyne* Project sponsored by USAID. Dr. Eisenback is currently a Professor of Plant Pathology at Virginia Tech where he serves as Director of the Virginia Tech Nematode Diagnostic and Assay Laboratory and teaches graduate courses in Plant Nematology and Seminar. Jon's research concentrates on nematode morphology and taxonomy, especially the root-knot nematodes, of which he has published numerous refereed research articles and book chapters. He has received international recognition for his winning photomicrographs. Dr. Eisenback has been honored

by the Society of Nematologists with two awards for his scientific research, the "Best Student Paper Award" and the "Ciba-Gigy Award for Outstanding Contributions to Agriculture." He has served the Society of Nematologists as Executive Board Member, Secretary, President, Archivist, and Nematology Newsletter Editor. Dr. Eisenback has served on the Board of CAST, the Board of the N.A. Cobb Nematology Foundation, the Society Presidents on the Board of Agriculture of the National Academy of Sciences, and was selected as a EUMANE scholar by the University of Ghent in Belgium.



### Ray Gill

Ray Gill received a B.S. degree as an Economic Entomology major in 1963 at the University of California, Davis. While at Davis he worked as a lab assistant for three years in the Entomology Department. After graduation he went to work as an Economic Entomologist and Taxonomist for the Riverside County California Office of the County Agricultural Commissioner from 1964 to 1971. Duties involved primarily systematics, taxonomy and identification of pest insects, survey and detection Entomology, plant quarantines and insect injury and plant disorder diagnosis. An M.S. degree was received from the University of California Riverside Department of Entomology while working for the Ag Commissioner. In 1971 he moved to Sacramento, California where he worked until 2004 on insects primarily in what was once called the "Homoptera." There he worked as a general taxonomist, Insect Biosystematist and eventually a Program Supervisor in the lab. Later his work was deeply involved with the *Bemisia tabaci* whitefly 'outbreaks' and has been involved in the publication of numerous articles on this subject. His interests have been scale insect, whitefly and leafhopper taxonomy, use of molecular techniques in taxonomy, insect induced plant injury, survey and detection techniques and plant quarantine issues. Just prior to retirement, he was partly involved in the fledgling development of NPDN through the University of California, Davis.



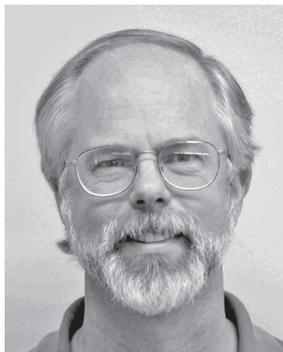


### **Carrie Harmon**

Carrie Harmon directs the hub laboratory of the SPDN, and leads a research, teaching, and extension program that focuses on disease detection, diagnosis, and training. She received her B.S. in Plant and Soil Science at the University of Massachusetts in 1999 and her M.S. in Plant Pathology at Purdue University in 2002. During her Master's study, Carrie started her diagnostic journey under the guidance of Karen Rane and Gail Ruhl in the Purdue Plant and Pest Diagnostic Laboratory, taking that experience with her to the University of Florida in 2003. She joined the faculty of the Department of Plant Pathology and served as the Associate Director of the SPDN under Gail Wisler, just as NPDP was getting started. Carrie was appointed Director of the UF Plant Disease Clinic in 2009, coupling the hub lab duties with coordination of the SPDN project. She broadened the scope of the clinic over the next several years, adding research and training to the outputs of the program. With the movement of the laboratory function into new space in 2013, she officially titled the program the Plant Diagnostic Center to encompass all three areas: teaching, research, and extension. Carrie now teaches three courses in plant disease diagnosis and applied disease management, provides hands-on diagnostic training to traditional students and professionals, and conducts research to improve plant disease detection and diagnosis. The SPDN hub laboratory processes nearly 3,000 samples per year, and serves a wide variety of clientele from around the world.

### **Charla Hollingsworth**

Charla Hollingsworth received her B.S., M.S. and Ph.D. degrees from the University of Wyoming in Laramie. Immediately thereafter, she established research and extension programs in small grains plant pathology in the Red River Valley of Minnesota where she was a faculty member at the University of Minnesota. She next accepted a position with USDA APHIS Plant Protection and Quarantine (PPQ), Science and Technology, Center for Plant Health, Science and Technology (CPHST) located in Raleigh, NC where her snow shoes hang unused on the wall. As a National Science Director for PPQ, she is responsible for providing scientific support to PPQ plant pathogen programs, and works closely with the National Plant Protection Lab Accreditation Program (NPPLAP), as well as the CPHST-Beltsville and CPHST-Mission labs.



### **Frank Martin**

Dr. Frank Martin has been a Research Plant Pathologist with the USDA-ARS in Salinas, CA since 1996. He received his Ph.D. in Plant Pathology from the University of California, Berkeley and was with the Plant Pathology Department at the University of Florida for 10 years prior to his current position. His research has focused on the ecology, biology, detection, identification and phylogeny of the genera *Pythium* and *Phytophthora*. Current approach is to use comparative analysis of mitochondrial genomes for identification of loci that are useful for designing highly specific TaqMan and isothermal markers in a systematic fashion. Development of technology that enables rapid analysis to be conducted at the point of sample collection is a primary objective of his research program.

### **Deborah G. McCullough**

Deborah G. McCullough holds graduate degrees in Forestry (M.S., Northern Arizona University) and Entomology (Ph.D., University of Minnesota). She is a Professor with a joint appointment in the Dept. of Entomology and Dept. of Forestry at Michigan State University, with research, extension and teaching responsibilities. Much of McCullough's current research focuses on the ecology, impacts and management of invasive forest insects, including emerald ash borer and beech bark disease. She works with forest management agencies, regulatory officials and private landowners to identify damaging forest insect populations and to develop long-term, sustainable management strategies to protect forest health. McCullough has published more than 100 papers about forest insect ecology and management in scientific journals, along with more than 200 extension bulletins and articles. She teaches Insects and Diseases of Forest and Shade Trees annually, to students majoring in Forestry or Horticulture.



### **Nancy Osterbauer**

Dr. Nancy Osterbauer is the Plant Health Program Manager with the Oregon Department of Agriculture in Salem. She received her B.A. in biology from the College of St. Scholastica, her M.S. in plant pathology from the University of Minnesota, and her Ph.D. in botany and plant pathology from Oregon State University. The Plant Health Program provides official field inspections and laboratory testing for Oregon's specialty seed, grass seed, and nursery industries. For other commodities, such as potatoes and onion, surveys to establish pest-free status for the state or for specific counties are conducted. These inspections, surveys, and lab tests are required for shipment to interstate and international markets. The Plant Health Program is also responsible for several state quarantines, control area orders, and other regulations for plant pathogens. These administrative rules are designed to prevent exotic pathogens from being introduced or becoming established in the state and to provide quarantine pest-free production areas for Oregon growers. Much of Dr. Osterbauer's research is focused on developing or improving diagnostic methods for pathogens of regulatory concern and on applying the systems approach to mitigate the risk of spreading pathogens through the shipment of plants for planting.



### **Megan Romberg**

Megan has been in the position of National Specialist in Mycology for USDA-APHIS since October of 2012, and provides final identifications for fungi on products arriving at US ports, and final confirmations for new-to-the US fungi from throughout the US. From 2008 to the end of 2011, Megan was a scientist at the Plant Health and Environment Laboratory (PHEL) of the Ministry for Primary Industries in New Zealand. In this position she provided the final identification of fungi and bacteria associated with plant products and other imports arriving in New Zealand. Samples processed at PHEL arrived from air and sea cargo, post entry quarantine and general surveillance from throughout the country. Prior to her time in New Zealand, Megan was a AAAS Science and Technology Policy Fellow at the USDA Economic Research Service where she provided biological input and information for economists studying phytosanitary barriers to trade. She has also spent multiple semesters teaching introductory biology at various community colleges in the Washington DC area. Megan earned her PhD in 2005 at the University of California, Davis where her research focused on diseases of potato and tomato.

### **William Schneider**

Dr. Schneider works as a Research Plant Pathologist for the USDA Agricultural Research Service with the Foreign Disease Weed Science Research Unit at Fort Detrick, MD. His educational background includes a BS from the University of Minnesota-Duluth in Biology and a Ph.D. from Michigan State University in Genetics. Following his Ph.D. Dr. Schneider took a post-doctoral fellowship with the Noble Foundation in Ardmore, OK. Dr. Schneider's expertise lies in the fields of virology, plant pathogen diagnostics and pathogen evolution. His current research program includes work on broad range diagnostics for plant pathogens, including pathogen detection arrays, PCR/ESI-MS and E-probe Diagnostic Nucleic acid Analysis (EDNA) of metagenomes and metatranscriptomes. The laboratory studies the biological

and evolutionary relationship between multiple high impact tree crop viruses and the insects that transmit them. He also studies the evolution of insect transmitted bacteria and the biology of the select agent bacterial pathogen *Rathayibacter toxicus*.



### **Steven Seybold**

Steven Seybold, Ph.D., is a Research Entomologist (GS-15) with the USDA Forest Service Pacific Southwest Research Station and a Faculty Affiliate and Lecturer in the Department of Entomology and Nematology at UC Davis. He is also a Contributor and Affiliate (2010 – present) to the Center for Invasive Species Research, UC-Riverside (<http://cistr.ucr.edu/faculty.html>) and a Member (2012 – present) of the UC-Davis Forest Biology Research Center (<http://dendrome.ucdavis.edu/fbrc/>). He has over 30 years of research experience in forest entomology, specializing in the study of bark and wood-boring beetles. He and his colleagues are characterizing the invasive bark beetle and woodborer fauna of California and other Western U.S. States. His lab develops lures for the detection of invasive



species and discovers and demonstrates the efficacy of behavioral chemical tools for the management of these forest pests. He received his B.S., 1983, in Forestry from the University of Wisconsin, Madison, and his Ph.D., 1992 in Entomology from the University of California, Berkeley. He has published over 90 scientific articles in refereed journals and 10 chapters in books on the topics of the origin and use of pheromones to sample bark beetles and other forest insects and on the biology and management of invasive forest insects. His most notable and highly cited research publications include the first demonstration of *de novo* pheromone biosynthesis in bark beetles (*Proc. Nat. Acad. Sci. USA* 92:8393–8397), a geographic study of pheromone distribution in a pine bark beetle (*JCE* 21:995–1016 and *Proc. R. Soc. Lond. B.* 266:1843–1850), and a review of isoprenoid (terpene) biosynthesis in pine bark beetles (*Ann. Rev. Entomol.* 48: 425–453). He has also published 45 non-refereed reports and 44 extension bulletins on the biology and management of bark beetles and woodborers. He is a member of the Entomological Society of America, the Entomological Society of Canada, and the International Society of Chemical Ecology.

### Christine Smart

Christine Smart has been a vegetable pathologist at Cornell University since 2003. Her lab focuses on the biology and management of vegetable diseases caused by bacterial and oomycete pathogens. She works with conventional and organic growers to detect, identify and reduce losses caused by pathogens. Additionally, she uses genomic tools to better understand pathogen populations and how those populations change over time.



### Jason Smith

Jason is the Co-Director Emerging Threats to Forests Research Team, Associate Professor of Forest Pathology and State Forest Health Extension Specialist at the University of Florida. The focus of his research program is to provide a better understanding of the underlying mechanisms and biology of interactions between tree hosts, pathogens, their vectors and the environment to reduce the impact of disease on trees in the context of global change. Current research focuses on 1.) Exotic tree disease detection and management; 2.) Phylogeography and pathogenicity of tree pathogens; and 3.) Management of diseases affecting rare, endangered or relict tree species in a changing climate. In addition to serving as the Principal Investigator of the Forest Pathology Laboratory, Jason teaches several graduate and undergraduate courses and carries out

forest health extension activities including advanced tree diagnostic services. Professionally, Jason is active in the American Phytopathological Society, American Conifer Society and serves as associate editor of the journal *Forest Pathology*.



### Les Szabo

Les Szabo directs an internationally recognized research program that focuses on the genomics, molecular diagnostics and population genetics of the wheat stem rust pathogen, *Puccinia graminis* f. sp. *tritici*. He received his B.S. in Biology from Washington State University, M.S. in Biochemistry from Michigan State University, and Ph.D. in Plant Pathology and Botany from Oregon State University. Following his Ph.D. he received Post-Doctoral training in fungal and plant molecular biology at Rockefeller University. He joined the USDA-ARS Cereal Disease Laboratory in 1988 and also holds Adjunct Faculty appointment in the Department of Plant Pathology, University of Minnesota. His laboratory has developed a number of DNA based molecular tools for rapid diagnostics and monitoring rust pathogens. This work has included monitoring aerial movement of soybean rust pathogen spores using a network of

wet deposition traps, development of qPCR assays for southern corn rust pathogen (*Puccinia polysora*) and the three wheat rust pathogens (*P. graminis*, *P. striiformis* and *P. triticina*). With the recent wheat stem rust epidemics in Africa his laboratory has developed qPCR assays for the *P. graminis* f. sp. *tritici* “Ug99” and TKTF race groups. Working with Borlaug Global Rust Initiative, his group leads the molecular diagnostics component of the international surveillance wheat stem rust pathogen.



### Sue A. Tolin

Sue A. Tolin received her B.S. (1960) from Purdue University, and M.S. (1962) and Ph.D. (1965) from the University of Nebraska-Lincoln. She then joined the plant pathology faculty at Virginia Tech, and retired as Professor Emerita in 2010. As a part-time IPA with USDA-CSRS (1978–1992), she contributed to biotechnology policy development, representing USDA on the Recombinant DNA Advisory Committee and international forums. Her academic research career focused on virus-host interactions and plant virus diagnostics, emphasizing RNA viruses of annual field crops, forage legumes, tobacco, vegetables, and perennial fruit crops. She has over 350 refereed journal articles, book chapters, reviews, and invited presentations. With breeders and geneticists, she investigated resistance of soybean to *Soybean mosaic virus* through classical and molecular genetics, identifying and mapping three resistance genes to the soybean genome. She directed research on plant viruses in developing countries for the US-AID IPM-CRSP (2005–2014), focusing on developing diagnostic capacity, vector and seed transmission, and ecologically-based disease management strategies. She taught plant virology, molecular plant-microbe interactions, translational plant science, and professionalism, including biosafety, biological compliance, and biosecurity. She has been an invited participant in conferences and work groups related to biosecurity, threat assessment and recovery plans, plant pathogen forensics, microbial genomics, emerging pathogens, invasive species, and biosafety. She is past president of the American Phytopathological Society (1994–1995) and served on several APS committees including the Public Policy Board (1991–2002) and Awards and Honors (2012–2016). She is a Fellow of APS, AAAS, and the American Academy of Microbiology.



### Ann Vidaver

Dr. Vidaver is a Professor emerita in the Department of Plant Pathology, University of Nebraska-Lincoln. She was Head of the Department (1984–2006), served as Director of the Center for Biotechnology (1997 to 2000) and was Chief Scientist for the USDA’s National Research Initiative Competitive Grants Program (2000–2002).

She started at Brookhaven National Laboratory before UNL. She has particularly served the American Phytopathological Society, including the Presidency. She is in the APS Press book on Pioneering Women in Plant Pathology. She has served as President of the Intersociety Consortium for Plant Protection, on the Board of Directors for the USDA’s Alternative Agricultural Research and Commercialization Corporation, on the Environmental Literacy Council, on the National Science Advisory Board for Biosecurity, and Federal agencies, including the NIH-RAC (Recombinant DNA Advisory Committee) and USDA-ABRAC (Agricultural Biotechnology Research Advisory Committee). She was Board President for H.A.W. Institute for Alternative Agriculture, chaired the Food and Agriculture Committee of the American Society for Microbiology’s Public and Scientific Affairs Board, and was a member of the USDA’s National Agricultural, Research, Extension, Education, and Economics Advisory Board. Research interests have focused principally on plant-associated bacteria, including systematics, epidemiology and control, plasmid, bacteriophage and bacteriocin characterization and genetics. She has contributed to research policy issues, including National Research Council publications. She has authored or co-authored over 200 scientific articles and a book, and holds two patents.

### Fred Warner

I am a Nematologist within Diagnostic Services at MSU. Essentially, I spend the majority of my time at the microscope identifying and counting microscopic roundworms. I assist clients/growers in management of plant-parasitic nematode problems if detected. I have served in this capacity for over 28 years.

I received my B.S. and M.S. degrees from Michigan State University majoring in Entomology. I came to MSU in the fall of 1975 and like thousands of other students wanted to get into vet school. I often get asked, “How did you get into Nematology?” I think part of the reason



is that while I worked at a veterinary clinic in high school, I gained a bit of a fascination with parasites. In addition, identification of insects and nematodes came quite easily to me and I recognized it as a potential strength.

If you detect an unusual accent when I speak, it's due to the fact I was born and raised in Massachusetts and will probably never lose the accent completely. I'm married with two kids, and currently live with my wife, a dog and a cat in Dimondale, MI.



### **Weimin Ye**

Dr. Weimin Ye was born in Jiujiang, Jiangxi, China in March 8, 1965. He received a B.S. from Department of Plant Protection, Jiangxi Agricultural University, Nanchang, China (1986), MSc from Department of Plant Pathology, South China Agricultural University, Guangzhou, China (1989), Department of Morphology, Systematics and Ecology, University of Gent, Belgium (1996) and Ph.D. from Department of Plant Pathology, University of Arkansas, USA (2002). His dissertation focused on taxonomy and molecular systematics of Longidoridae nematodes from USA. He was a Post Doctoral Fellow in Purdue University and University of New Hampshire. He worked in Shenzhen Animal & Plant Quarantine Bureau, Shenzhen, Guangdong Province, China (1989–1998) and Fort Lauderdale Research and Education Center, University of Florida. He joined Agronomic Division, North Carolina Department of Agriculture and Consumer Services, Raleigh, North Carolina from 2005 and is an Adjunct

Assistant Professor, Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina from 2009. He is a past Chair in Nematode Systematic Resources in Society of Nematologists and is the 2015 recipient of the award for Research Excellence in the field of Nematology presented by Society of Nematologists and Syngenta dedicated to early-career scientist who has made significant contributions to the advancement of Nematology and Agriculture.



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SPDN, University of Georgia

**Len Coop**  
WPDN, Oregon State University

**Tom Creswell**  
NCPDN, Purdue University

**Marty Draper**  
USDA-NIFA

**Mike Hill**  
CERIS, Purdue University

**Paul Jepson**  
WPDN, Oregon State University

**Eileen Luke**  
CERIS, Purdue University

**Neil McRoberts**  
WPDN, University of California,  
Davis

**Forrest Nutter**  
NCPDN, Iowa State University

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## SENTINEL PLANT NETWORK WORKING GROUP

**Marc Fuchs**

*NEPDN Director, Cornell University, Project PI*

**Rachel McCarthy**

*NEPDN, Cornell University, NPDN-SPN Manager*

**Rick Bostock**

*WPDN Director, University of California, Davis*

**Marty Draper**

*NIFA Program Leader, USDA-NIFA*

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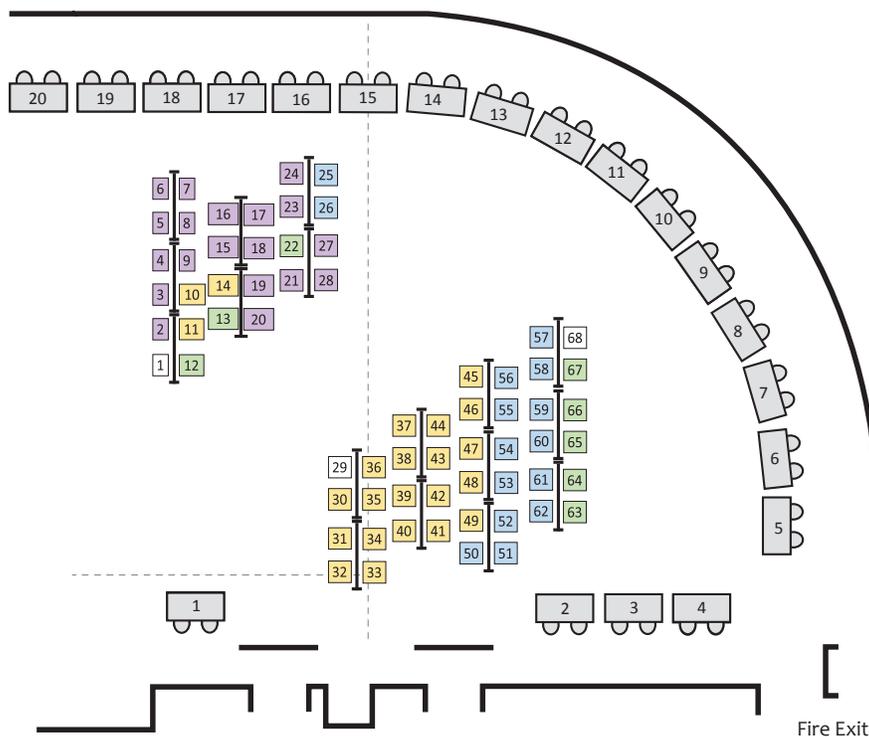




## CRYSTAL BALLROOM LAYOUT

**POSTER TOPICS**

- Emerging Pathogens and Pests
- Advancing Diagnostics Partnerships and Projects
- NPDN's Role in Advancing Diagnostics
- Novel Methods to Improve Prevention, Detection and Diagnosis for Food Security and Trade



## EXHIBITOR LISTINGS

Table #	Organization	Name	Website
1	STAR-D Laboratory Accreditation	Dawn Dailey O'Brien	
2	Gylling Data Management	Fran Gylling	<a href="http://www.gdmdata.com">www.gdmdata.com</a>
3	PathSensors, Inc. (PSI)	Forrest Miller	<a href="http://www.pathsensors.com">www.pathsensors.com</a>
4	Sentinel Plant Network	Rachel McCarthy	<a href="http://www.sentinelplantnetwork.org">www.sentinelplantnetwork.org</a>
5	Distance Diagnostics through Digital Imaging (DDDI)	Sherri McElroy Clark	<a href="http://www.dddi.org">www.dddi.org</a>
6	Martin Microscope Company	Robert Martin	<a href="http://www.martinmicroscope.com">www.martinmicroscope.com</a>
7	Percival Scientific, Inc	Patrea Hill	<a href="http://www.percival-scientific.com">www.percival-scientific.com</a>
8	Bioreba	Judit Monis	<a href="http://www.eurofinsus.com/sta-laboratories/bioreba-ag">www.eurofinsus.com/sta-laboratories/bioreba-ag</a>
9	Agdia, Inc.	Keith Schuetz	<a href="http://www.agdia.com">www.agdia.com</a>
10	Plant Diagnostic Information System (PDIS)	Judy Dizon	<a href="http://www.pdis.org">www.pdis.org</a>
11	Bartlett Tree Experts	Megan McConnell	<a href="http://www.bartlett.com">www.bartlett.com</a>
12	Regional IPM Centers	Rosemary Hallberg	<a href="http://www.ipmcenters.org">www.ipmcenters.org</a>
13	Conviron	Richard Marusyk	<a href="http://www.conviron.com">www.conviron.com</a>
14	Biolog	Stacy Montgomery	<a href="http://www.biolog.com">www.biolog.com</a>
15	OptiGene Limited	Michael Andreou	<a href="http://www.optigene.co.uk">www.optigene.co.uk</a>
16	USDA Plant Protection and Quarantine	Patrick Shiel	<a href="http://www.aphis.usda.gov/aphis/ourfocus/planthealth">www.aphis.usda.gov/aphis/ourfocus/planthealth</a>
17	Extension Disaster Education Network (EDEN)	Steve Cain	<a href="http://www.purdue.edu/extension">www.purdue.edu/extension</a>
18	Doctor of Plant Medicine/Health Program	Amanda Hodges	<a href="http://www.dpm.ifas.ufl.edu">www.dpm.ifas.ufl.edu</a>
19	Protect U.S. and NPDN Online Training Resources	Amanda Hodges	<a href="http://www.protectingusnow.org">www.protectingusnow.org</a>
20	Collaborative and Enhanced First Detector Training	Amanda Hodges	<a href="http://www.flfirstdetector.org">www.flfirstdetector.org</a>

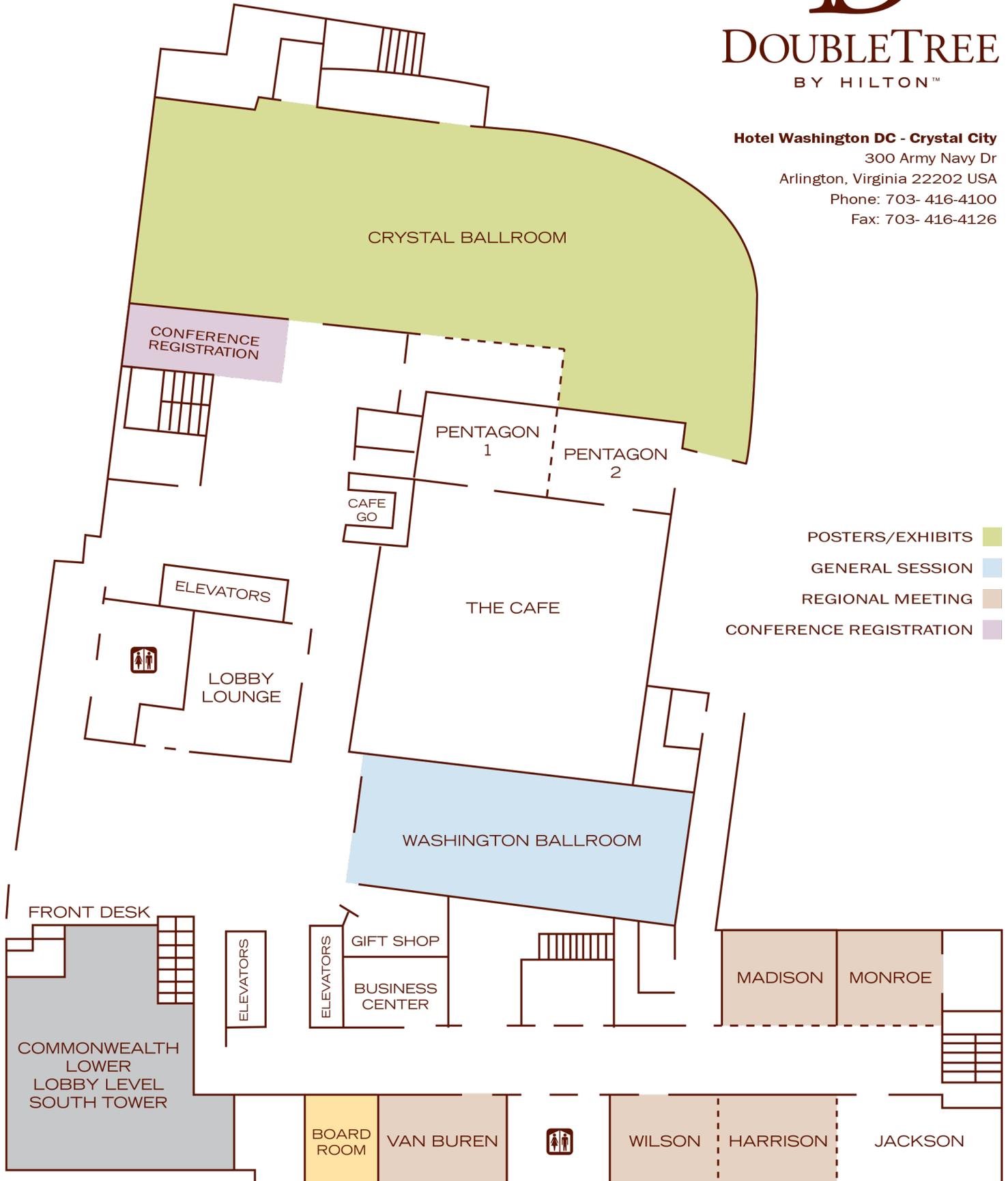


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