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Service

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Safeguarding Through Science

Center for Plant Health Science and Technology

2012 Accomplishments

Center for Plant Health Science and Technology

Mission

The Center for Plant Health Science and Technology (CPHST) supports the regulatory decisions and operations of the Animal and Plant Health Inspection Service's (APHIS) Plant Protection and Quarantine (PPQ) program through methods development, scientific investigation, analyses, and technology.

Strategic Goals

- Enhance PPQ's efforts in pest detection and management
- Provide timely scientific and technical support required for emergency response and management
- Enhance support for APHIS trade-related plant health issues
- Provide current, relevant scientific and technical information to PPQ decisionmakers
- Enhance PPQ's capacity to anticipate and respond to emerging scientific, technical, and regulatory issues through partnership

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Cover Photo: Pink bollworm larvae emerging from a cotton boll (*USDA/ARS, Peggy Greb*). In a project highlight, the CPHST Phoenix Lab describes a project that successfully demonstrated that strontium chloride can be used as a secondary marker for pink bollworm reared for the Pink Bollworm Eradication Program. Without an additional marker, reared moths could be misidentified as wild moths, resulting in costly eradication actions.

Message from the Executive Director

The Center for Plant Health Science and Technology (CPHST) provides scientific and technical support for the regulatory decisions, policies, and operations of the Animal and Plant Health Inspection Service's (APHIS) Plant Protection and Quarantine (PPQ) program in order to safeguard U.S. agriculture and natural resources. CPHST ensures that PPQ has the information, tools, and technology to make the most scientifically valid regulatory and policy decisions possible. CPHST also ensures that PPQ's operations have technically feasible and practical tools for pest exclusion, detection, and management. The 2012 CPHST Accomplishments Report is intended to offer an in-depth look at the status of our programs and the progress we have made toward the Center's mission and long-term strategic goals.

The organization includes approximately 245 employees in 7 labs, 4 programs, and multiple work units. Our scientists provide leadership and expertise in a wide range of fields—including pest risk assessments that support trade, commodity quarantine treatments, pest survey and detection methods, identification tools and molecular diagnostics, and integrated pest management.

The past year has been a time of transition for both PPQ and CPHST. In October 2012, PPQ reorganized into a new structure with three core functional areas: Policy Management, Field Operations, and Science and Technology (S&T). The goal of this reorganization is to help transform PPQ into a more flexible, efficient, collaborative, consistent, and risk-based organization.

CPHST is the main component of S&T, which also includes the National Clean Plant Network program. Concurrently with the PPQ reorganization, CPHST reorganized its leadership structure so that the Associate Director and six National Science Program Leader positions were consolidated into four Associate Directors. The Associate Directors have administrative responsibilities in addition to overseeing CPHST's portfolio of projects. The Associate Directors play a key role in providing a focal point for communications and coordination with stakeholders, customers, and the scientific and regulatory community, as well as strategic, operational, and fiscal planning.

In addition to these leadership changes, there were several changes in CPHST's laboratory structure in 2012.

- The CPHST Agricultural Quarantine and Inspection (AQI) Lab in Miami completed renovations and hired a laboratory director in early 2013. The Treatment Quality Assurance Unit was incorporated into the AQI Lab and is now CPHST AQI-Raleigh.
- The Gulfport Lab closed, and remaining staff relocated to the AQI Lab or the new Biloxi Station in Mississippi.
- The Light Brown Apple Moth Unit was renamed the California Station and relocated from Moss Landing to the USDA Agricultural Research Service station in Salinas. Much of the work conducted by station personnel now supports European grapevine moth and Asian citrus psyllid programs.
- The Biological Control Unit and the Fruit Fly Unit were renamed the Biological Control Program and Fruit Fly Program.

- In late 2011, the PPQ Molecular Diagnostics Laboratory merged operations with the CPHST Beltsville Lab. The Beltsville Lab is now responsible for conducting plant pathogen operational diagnostics as well as developing diagnostic techniques for regulated plant pathogens.
- CPHST established a National Scientific Technologies Program to assess and transfer detection, identification, diagnostic, and exclusion tools and technologies to support PPQ programs.

CPHST is recognized nationally and internationally for its leadership in scientific development to battle plant pests and diseases. We are pleased to issue this annual report and provide an informative overview of the Center's accomplishments and projects.

Philip Berger, Executive Director
Center for Plant Health Science and Technology
PPQ Science & Technology
USDA-APHIS

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PPQ Science and Technology

Organizational Chart

Laboratories and Programs

Office of the Executive Director, Raleigh, NC

Executive Director: Philip Berger

Associate Directors: Ron Sequeira, Michael Hennessey, Charla Hollingsworth, and Russ Bulluck

In October 2012, PPQ was restructured into three core functional areas: Policy Management, Field Operations, and Science and Technology (S&T). S&T is composed of CPHST and the National Clean Plant Network (NCPN) program. The leadership structure at CPHST headquarters was also reorganized and is now led by the S&T Executive Director and four Associate Directors. The S&T Office of the Executive Director provides administrative support to CPHST laboratories, coordinates with PPQ programs on project needs and priorities, and provides cross-laboratory program coordination. In addition, this office provides scientific input on initiatives within PPQ and represents PPQ on scientific issues and collaborations with other Federal agencies and partners.

Recent Accomplishments

- Supervised the completion of renovations at the CPHST Agricultural Quarantine and Inspection (AQI) Lab in Miami, FL. This lab will provide major support to the agency's AQI programs, as well as biocontrol and analytical and environmental chemistry support.
- Led and coordinated commodity treatment and inspection methods development across PPQ and with the U.S. Department of Homeland Security (DHS), USDA Agricultural Research Service (ARS), and international cooperators.
- Coordinated citrus health methods development work in support of PPQ operations and policy units, emphasizing discovery and implementation of effective management practices and diagnostic tools. Managed and reviewed 13 citrus-related cooperative projects for 10201 Farm Bill funding and 11 CPHST internal projects. CPHST addresses issues arising from established plant diseases such as *huanglongbing* (citrus greening; HLB), citrus canker, sweet orange scab, and citrus black spot, as well as diseases not known to occur in the United States such as citrus variegated chlorosis and *Citrus leprosis virus* (CiLV).
- Led the National Citrus Health Response Program Science and Technology Research Coordination Group. Coordinated citrus research efforts nationally and internationally and organized the annual Citrus Health Research Forum meeting that brings together researchers,

technical experts, and regulatory programs from across the United States. This effort supports open communication and coordination across the citrus research community to benefit scientific inquiry and inform stakeholders.

- Established a working group to coordinate research activities related to CiLV and its mite vector. The goal of this group, which includes national and international scientists, is to develop new tools and techniques for detecting and identifying the mite and virus.
- Coordinated research conducted in China targeting citrus greening disease and investigated integrated approaches to disease management. Working with cooperators at North Carolina State University (NCSU), CPHST continued to focus on outreach and sharing findings related to the role of nutritional approaches, the value of thermal therapies, and the potential importance of climate change in affecting citrus greening disease patterns. In 2012, ARS provided the first stateside confirmation of observations regarding the value of thermal therapies communicated by Chinese cooperators in 2010.
- Provided support for developing USDA climate change strategies. Specifically, CPHST led the formulation of plans to capture how climate change affects phytosanitary programs. CPHST offered input on how pest forecasting systems can be modified by using general circulation models linked to existing epidemiological systems to give updated predictions of pest distribution and impact.
- Led the evaluation of next-generation epidemiological and forecasting simulation models to inform regulatory decisions. CPHST has leveraged cooperative links between ARS, Michigan State University, Oregon State University, NCSU, and others to evaluate a suite of candidate products against known case studies. As a result of the first phase of evaluations, CPHST has identified alternative models, recognized information security needs, and developed a validation strategy.
- Provided support for the National Ornamentals Research Site at Dominican University of California (NORS-DUC). This is the only established field research facility in the country designed to study regulated plant pathogens in a secure environment. NORS-DUC focuses on Systems Approaches for Nursery Production and on developing science-based best management practices and risk mitigation to exclude, contain, and control the regulated pest *Phytophthora ramorum* from the nursery production chain through investigations conducted by a consortium of expert *P. ramorum* researchers.

- Led S&T efforts on emergency response and nursery and forest pest programs, including *P. ramorum* and potato cyst nematode.
- Represented PPQ on interagency groups focused on biosurveillance science and technology, such as the Biological Indications and Warning Analytic Community.

CPHST AQI Laboratory, Miami, FL and Raleigh, NC

Laboratory Director: Woodward Bailey

The newly-formed CPHST AQI Laboratory is colocated with ARS at the Subtropical Horticulture Research Station in Florida. In addition to its Miami location, the CPHST AQI Lab includes treatment support staff in Raleigh, NC. CPHST's Biloxi Station in Mississippi also reports to the AQI Lab and oversees imported fire ant (IFA) projects and pesticide residue analysis of environmental monitoring samples. The mission of the AQI Lab is to provide world leadership in developing phytosanitary measures and port technologies to protect American agriculture and natural environments from invasive pests. Specific goals include: developing new quarantine treatments and techniques and modifying existing methods that mitigate risks associated with quarantine pests; validating existing treatments; supporting PPQ Field Operations and Preclearance programs (such as facility approvals and certifications); developing risk management systems and systems approaches; and developing and optimizing port technologies and processes related to pest exclusion.

Recent Accomplishments

Treatment Schedule Development and Support

- Reviewed cold treatment research protocols for Mediterranean fruit fly (Medfly) in Spanish clementines and four species of fruit flies in Hawaiian citrus.
- Approved final efficacy data for irradiation of mango pulp weevil in Philippine mangoes and cold treatment of Queensland fruit fly in Australian grapefruit.
- Reviewed research and provided technical recommendations for the import of spinach seed with *Phomopsis* spp.
- Reviewed ethyl formate research for control of light brown apple moth (LBAM) on grapes.
- Reviewed research protocols for Asian citrus psyllid (ACP), citrus black spot, and citrus canker.
- Reviewed fruit fly development data as one component of a hot water immersion study on oversized Peruvian mangoes.
- Approved three spray schedules to disinfest Japanese beetle and other flying hitchhikers in cargo holds and containers to replace 10-percent d-phenothrin (no longer registered for use by the U.S. Environmental Protection Agency [EPA]).
- Compiled a document titled "USDA-APHIS Guidelines for Research Supporting Fruit Fly Cold Treatment Development" to standardize and identify criteria for treatment research and evaluation.

Technical Support for Field Operations and Preclearance Programs

- Approved Dupont Tyvek air cargo covers for covering pallets of perishable commodities during fumigation.
- Approved new options for placement of circulation fans in fumigation chambers.
- Approved modified atmosphere packaging for Mexican guavas.
- Reviewed protocol and provided guidance on Australian pilot project for in-transit cold treatment of mixed citrus consignments.
- Developed operational guidelines for sulfuryl fluoride fumigations of containerized logs.
- Approved plans and construction proposals for new types of fumigation chambers at South Florida Logistics Services.
- Certified and approved equipment, facilities, and conveyances for cold treatment of fruit: certified 123,438 self-refrigerated containers and 32 vessels; approved plans for three new vessels and/or significant alternations of existing vessels and one warehouse.
- Approved two international hot water mango facilities and equipment for mango treatment; approved changes to eight existing international hot water mango facilities.
- Developed new irradiation policies concerning process configuration approvals, calibration of dosimetry systems, and insect-proof packaging.
- Approved and certified two domestic irradiation facilities and recertified two international irradiation facilities.

Analytical Chemistry Support

- Developed a chemical assay for determining LBAM pheromone blend in mating disruption projects; evaluated 210 pheromone blend samples.
- Developed a chemical assay to determine d-phenothrin content in aerosol products for the Japanese Beetle Program; evaluated 4 aerosol product samples and 12 quality control samples.

- Evaluated four quality assurance samples for fruit fly programs.
- In conjunction with DHS, developed an elemental analysis test to determine the origin of mango imports.

Imported Fire Ant Program

- Initiated agreements with ARS and several universities to conduct IFA methods development work on treatments for grass sod and field-grown nursery stock.
- Coordinated phorid fly biological control releases with State cooperators.
- In conjunction with PPQ Plant Health Programs, Field Operations, and APHIS Legislative and Public Affairs, produced an industry alert for hay producers, buyers, and sellers to answer questions about moving hay from areas under IFA quarantine.
- In conjunction with the PPQ Professional Development Center, prepared language for the addition of two treatments to the IFA section in the PPQ Treatment Manual.

Analysis of Routine Environmental Monitoring Samples

- Between October 1, 2011 and March 15, 2012, analyzed 152 samples for the Boll Weevil, Asian Longhorned Beetle, and IFA programs.
- Established an interagency agreement with USDA's Agricultural Marketing Service (AMS) to analyze routine PPQ program environment samples with predetermined per sample costs; AMS began providing program service after March 15, 2012.
- Designed a new reporting system in conjunction with AMS and PPQ Plant Health Programs.
- Redesigned environmental sample supply request forms and trained PPQ samplers on new request and shipping procedures.

CPHST Beltsville Laboratory, Beltsville, MD

Laboratory Director: Mark Nakhla

The CPHST Beltsville Laboratory's mission is to develop, validate, and carry out advanced biochemical and molecular methods for the detection of high-consequence plant pathogens, including those on USDA's Select Agents and Toxins list and plant pathogens in foreign germplasm. In October 2011, the CPHST Beltsville Lab was merged with the existing PPQ Molecular Diagnostics Lab; as a result, the Beltsville Lab now also conducts operational diagnostics of plant pathogen samples.

Laboratory programs utilize cutting-edge technologies from the fields of plant pathology, molecular biology, human and animal clinical diagnostics, and bioterrorism to develop, adapt, and improve methods for the accurate and rapid diagnosis of plant pathogens. The laboratory also diagnoses and differentiates high-consequence and select agent plant pathogens that require Federal confirmation. The Beltsville Lab strives to achieve timely transfer of diagnostic tools that are field deployable for PPQ emergency response and eradication programs. The lab deploys these tools to stakeholders through clearly written standard operating procedures and hands-on laboratory training for end users within and outside of PPQ.

The Beltsville Lab is a key component of the PPQ National Plant Pathogen Laboratory Accreditation Program (NPPLAP). Beltsville is responsible for proficiency test panel development, delivery, and first-level evaluation conducted by scientists who perform diagnostics on behalf of PPQ using CPHST-validated methods. The laboratory is committed to quality in biochemical and molecular diagnostics and is proficiency tested in the operation of diagnostic methods. Beltsville staff conduct outreach to the plant pathology diagnostic community by providing technical support—protocols, hands-on laboratory training, and troubleshooting for PPQ-validated diagnostics—to scientists within the National Plant Pathogen Diagnostic Network (NPDN), PPQ port and regional identifiers, and the State departments of agriculture. Beltsville scientists also contribute their expertise in detecting regulatory plant pathogens by serving as members of scientific groups and committees.

Recent Accomplishments

- Developed a conventional polymerase chain reaction (PCR) assay to detect the Nepovirus Subgroup B for germplasm screening.
- Characterized a novel virus causing symptoms similar to citrus leprosis and proposed the virus be called *Citrus leprosis virus* cytoplasmic type 2.
- Evaluated an enzyme-linked immunosorbent assay (ELISA) for detecting *Citrus leprosis virus*-C.
- Developed assays to differentiate the sweet orange fungal pathogen, *Elsinoë australis*, subgroups and species.
- Developed conventional and quantitative PCR molecular diagnostics for the late wilt of corn fungal pathogen, *Harpophora maydis*.
- Developed molecular markers for differentiating isolates of *Phytophthora kernoviae* from Great Britain and New Zealand.

- Developed molecular assays for detecting and differentiating pathogenic nematodes *Anguina funesta*, *A. agrostis*, *A. tritici*, and *A. pacificae*. Morphological identification is difficult and labor intensive, and these assays provide a simpler method to differentiate these species.
- Validated real-time PCR methods for detecting and identifying *Rathayibacter toxicus* (a toxigenic bacterium vectored by the nematode *Anguina funesta*), which can cause disease in livestock that eat infected ryegrass.
- Developed assays to detect two additional genes of *Candidatus Liberibacter asiaticus* associated with citrus HLB.
- Evaluated and adapted CANARY technology (Cellular Analysis and Notification of Antigen Risk and Yield) for the rapid detection of *Phytophthora*. The optimized *Phytophthora* CANARY system was used to successfully detect field-collected and laboratory-inoculated samples.
- Evaluated and adapted the Lincoln Nucleic Acid Kit (LiNK) technology for the rapid extraction of plant pathogen DNA. The LiNK sampling methods were highly sensitive with a processing time of only 6 minutes. This system will be further optimized for use in plant pathogen diagnostics.
- Prepared proficiency test panels for HLB/citrus greening, *Plum pox virus*, and *P. ramorum* for the NPPLAP.
- Completed and released nine new or revised work instructions documents. These covered a diverse range of diagnostic methods, training instructions, and DNA/RNA isolation protocols.
- Provided five hands-on training sessions on diagnostics and bioinformatics for 42 scientists and diagnosticians from PPQ, NPDN, and several universities.
- In fiscal year (FY) 2012, completed regulatory diagnoses for 13 different plant pathogens, testing a total of 1,606 individual samples. Of these samples, 1,455 were negative, 92 were positive, and 39 were inconclusive for the pathogen of concern. Twenty samples were part of a project with ARS. Between June and September 2012, processed an unprecedented 1,290 *Plum pox virus*-suspect samples.

CPHST Fort Collins Laboratory, Fort Collins, CO

Laboratory Director: Richard Zink

Work at CPHST's Fort Collins Laboratory focuses on five critical areas of methods development for APHIS: pest identification technologies, pest survey protocols

and guidelines, risk mapping, AQI waste disposal and decontamination, and biological control. The laboratory provides extensive scientific support for the Cooperative Agricultural Pest Survey (CAPS) program. The laboratory transfers methodologies and tools to field operations to ensure efficient and effective survey, detection, identification, emergency response, and eradication efforts. The Fort Collins Lab also develops electronic, matrix-based identification resources to help support rapid, consistent, and accurate identification and nomenclature of pest species.

The Fort Collins Lab delivers innovative and cost-effective methods for managing invasive plants on public lands through insect biological control agents and chemical and cultural control procedures. PPQ operational programs receive spatial technology support from the laboratory to guide them in the application of new geospatial survey and detection methods. At a satellite location in Albany, CA, the laboratory's efforts continue to improve upon artificial diets for rearing insects for the biological control of weeds and for use in eradication programs, such as pink bollworm (PBW). Staff at the Albany location also develop protocols for biochemical analysis and identification of wild and artificially reared insects.

Recent Accomplishments

Rearing of the Yellow Toadflax Stem-Mining Weevil

- Continued a greenhouse-based rearing program for the stem-mining weevil *Mecinus janthinus*, a biocontrol agent of the exotic weed yellow toadflax (*Linaria vulgaris*). In 2012, produced more than 800 adult weevils and refined rearing methodologies. While weevils were not released in the field in 2012, they were used to increase production of the colony for 2013. The rearing program is designed to supplement field collections of *M. janthinus* at Montana sites, where populations are decreasing due to host plant mortality caused by the weevil.

Asian Gypsy Moth Trapping Model for Texas

- Delivered a geospatial model to identify highest introduction risk areas for Asian gypsy moth (AGM, *Lymantria dispar*). The State of Texas has a comprehensive surveillance system in place to identify (and eradicate, if necessary) any moths prior to establishment. This effort assisted 2012 AGM trap allocation for Texas and provided new information on where

AGM could be introduced and may establish. The model is based on a combination of these pathways and uses a variety of human-mediated and environmental variables. The work is now being used to inform a larger modeling effort for both European and Asian gypsy moth at a national scale.

Database of Historical Barberry Survey Data

- Continued development of a geospatial database to support the Black Stem Rust/Barberry program. The database has grown to include historical barberry eradication records from nine States (Idaho, Montana, Washington, Oregon, North Dakota, South Dakota, Wyoming, Minnesota, and Wisconsin). Overall goals of the project are to: (1) create a permanent electronic archive of historical survey data for visualization and query; (2) use the archive to develop tools for early warning of any new races of the rust pathogen appearing in wheat-growing regions; and (3) assist in current barberry survey efforts by directing staff to where the plant existed previously.

Weather Models Comparison Analysis

- Delivered an analytical report comparing the statistical accuracy of degree-day calculations from four weather modeling platforms against an independent weather data source. The weather modeling platforms evaluated were NAPPEFAST Modeler, NAPPEFAST Degree Day Analyst, BioSim, and USPest.org. The goal of the report was to identify and evaluate other providers and resources for weather data and degree-day models. The report also includes comparison tables on software cost, accuracy, reliability, ease of use, and timeliness.

Assessment of Disinfectants for Control of *P. ramorum*

- Under a cooperative agreement with Colorado State University, conducted three greenhouse studies to determine the phytotoxicity effects of oxidant disinfectants on nursery plant foliage. The study showed that two commercial disinfectants did not significantly injure plant foliage after repeated applications and may even improve the physiology of foliar gas exchange rates, which could be used in nurseries to decontaminate plants exposed to *P. ramorum* spores.

CAPS Support

- Released the “Cotton Commodity-Based Survey Reference and Guideline” and the “Asian Defoliator Pathway-Based National Survey Reference.”

- Released pest datasheets for eight insect, nine plant pathogen, and four nematode pests. Datasheets were also revised for seven insect and one mollusk pest.
- Codeveloped the *Cerceris* Wasp Survey Protocol and a survey protocol for detecting kiwi canker.
- Developed outreach documents on *Tuta absoluta*, *Neoleucinodes elegantalis*, *Adoxophyes orana*, *Empoecilia ambigua*, and *Spodoptera littoralis*.
- Provided support to CAPS staff on survey and diagnostic information requests.

Identification Technology Program Releases

- Flat Mites of the World*, CPHST’s most popular tool to date with over 55,000 unique visitors in 2012 (<http://idtools.org/id/mites/flatmites>)
- Citrus Resource*, CPHST’s first complete commodity-based resource, including image galleries, factsheets, and keys to pests, disease symptoms, and citrus cultivars (www.idtools.org/id/citrus/resource)
- TortAI*, CPHST’s first tool to identify tortricid moth adults and larvae to support national port interceptions and domestic surveys undertaken by the CAPS community (<http://idtools.org/id/leps/tortai>)
- Antkey*, CPHST’s first tool developed in Scratchpads, a social networking platform that allows communities to bring taxonomic information together in a media-rich and user-friendly Web site for specialists and nonspecialists (www.antkey.org)

Identification Technology Program:

International and Domestic Partnerships

- Collaborated with academic institutions from 11 States to develop numerous Web-based products for use by PPQ and State cooperators in identifying plant pests.
- Hosted, through the European Phytosanitary Research Coordination group, a delegation of plant protection scientists from Ukraine who came to the United States to learn how to build a program similar to the Identification Technology Program (ITP).
- Collaborated with three international experts from Australia, Belarus, and Canada who lent their taxonomic expertise to develop identification tools on mites (<http://idtools.org/id/mites/flatmites>), leaf beetles (<http://idtools.org/id/beetles/diabrotica>), and aphids (<http://aphid.aphidnet.org>), respectively.
- In spring 2012, released ITP’s new pest image library, IDpic Image Node, which was accessed by the global public over 191,000 times by the end of the year.

- By repurposing images and content from two of ITP's 2011 Web sites (www.idtools.org/id/citrus/diseases and www.idtools.org/id/citrus/pests), the University of Florida developed smartphone mobile applications (apps) for homeowners, farmers, and industry members interested in identifying citrus pests and diseases.

CPHST Mission Laboratory, Edinburg, TX

Laboratory Director: Matthew Ciomperlik

The CPHST Mission Laboratory supports PPQ's programs by developing pest detection and management methods, mitigation strategies, and molecular diagnostic tools for invertebrate pests. The laboratory identifies, develops, and transfers a wide range of scientific methods to PPQ and State departments of agriculture. In addition to these core functions, the laboratory cooperates with stakeholders and researchers in offering expertise to PPQ on the epidemiology of plant diseases, remote sensing/geographic information systems, biological control, areawide pest management, and sterile insect technology (SIT) support for the Mexican Fruit Fly Eradication Program.

In providing scientific expertise to PPQ on technological advances in molecular biology, the Mission facility has developed advanced strengths in the evaluation and application of DNA technologies and bioinformatics tools. This includes cutting-edge methods used for DNA barcoding of pest species to confirm identifications, microsatellite analysis of pest populations to track high-risk pathways of invasive species, and screening of insect genomes to develop new molecular tools. These in-house techniques and skills support PPQ programs through the development of identification tools and integrative projects to understand pest and vector distributions, dispersal, introduction pathways, and behaviors.

Recent Accomplishments

Asian Citrus Psyllid/Huanglongbing

- Developed maps, data, and analysis to support HLB survey efforts and technical working groups in understanding the discovery of citrus greening disease in Texas. Continued support of the Texas citrus industry with maps and data on commercial citrus production in the Lower Rio Grande Valley.
- Released over 117,000 adult *Tamarixia radiata* parasitoids in 140 locations in the Rio Grande Valley of Texas. Releases

targeted the area within a 5-mile radius of where citrus greening disease was detected in 2012. Establishment of *T. radiata* was confirmed at 11 release sites in 2012. Parasitism levels were measured at 10.4 percent, and estimated host-feeding damage was 64.9 percent.

- Transferred in-field insectary mass-rearing methods to the University of Florida, University of California-Riverside, California Department of Agriculture (CDEA), and PPQ Puerto Rico Work Unit.
- In response to a stakeholder inquiry, conducted experiments that showed lighted sticky traps would not be effective to detect ACP in shipments of citrus entering the United States from Mexico.

Mexican Fruit Fly Sterile Insect Technique

- Conducted quality control testing of Guatemalan black pupa (male predominate) strain, including mating compatibility and competitive testing against three Texas fly strains.
- Identified causal agent bacteria (*Morganella spp.*) for mortality to larval instars from eggs shipped from Guatemala and developed sanitation methods to overcome the problem.
- Conducted sampling of brush lands and harvested citrus groves to identify potential over-summering source locations for fertile fruit flies.
- Evaluated and calibrated Mexican fruit fly (Mexfly) aircraft and ground release machines.
- Evaluated effectiveness of two-component lures and torula yeast used in trapping.
- Constructed a Mexfly monitoring tool, allowing fruit fly coordinators and decisionmakers to analyze the effectiveness of sterile releases in the Mexfly program areas in Texas.
- Organized a fruit fly meeting at the CPHST Mission Laboratory to discuss diagnostic capacities and needs of USDA and State programs. The meeting included fruit fly experts from California, Florida, Hawaii, Texas, and Washington, DC. As a result of the meeting, the Tephritidae Diagnostics Group was formed, and CPHST initiated several collaborative efforts with USDA-ARS Hawaii, CDEA, and the Florida Department of Agriculture and Consumer Services (FDACS).

Molecular Diagnostics

- Finalized development of a repository of DNA isolates representing HLB-symptomatic leaf material from quarantined, HLB-positive citrus groves in the Rio Grande

Valley of Texas. The DNA isolates are available upon request to U.S. HLB researchers.

- Documented mitochondrial genetic diversity in and among populations of ACP (*Diaphorina citri*). This work tests new hypotheses regarding recent gene flow into U.S. populations of *D. citri*. In addition, identified useful microsatellite markers and established multiplex reaction panels to evaluate these markers efficiently. This work is being used to evaluate *D. citri* genetic variation at a much-reduced cost.
- Completed description of *Colosinus confusus*, a slug species originating from South America that is commonly intercepted at U.S. ports of entry (based on morphology and molecular evidence). This project was conducted to support PPQ-National Identification Services (NIS).
- Completed a molecular survey of amber snails at the USDA Plant Inspection Station in Los Indios, TX, to support PPQ-NIS. The study revealed that more than one species of *Calcisuccinea* is intercepted on commodities from Mexico. The hypothesis that *Calcisuccinea luteola*, a species native to Texas, was the only species being intercepted at this Plant Inspection Station was not accepted.
- Completed an evaluation of Cuban slug (*Veronicella cubensis*) diversity and, with PPQ-NIS, developed recommendations to enhance PPQ identification practices of this pest using molecular and morphological characters.

CPHST Otis Laboratory, Otis Air National Guard Base, Buzzards Bay, MA

Laboratory Director: Vic Mastro

The CPHST Otis Laboratory's mission is to identify, develop, and transfer technology for survey, exclusion, control, and risk assessment on behalf of APHIS and its cooperators. The lab serves a wide variety of PPQ programs, including exotic pest detection and phytosanitary treatments. The lab also supports emergency response and eradication programs for Asian longhorned beetle (ALB), European grapevine moth (EGVM), AGM, emerald ash borer (EAB), *Sirex noctilio* woodwasp, and other pests. Otis personnel identify high-risk exotic pests and develop survey technology to facilitate early detection of introductions. The lab continues to support the AGM program by developing molecular methods to distinguish among subspecies of gypsy moth; producing the gypsy

moth virus product, Gypchek; and helping ensure the quality of gypsy moth lures. Recent developments in the use of microsatellites is better defining the geographic distribution of gypsy moth strains throughout the world. Additional work is focused on developing regulatory treatments for various commodities and their means of conveyance, such as pallets and containers. The Otis Lab is developing rearing systems for EGVM and ALB and has biological control programs for EAB, *Sirex noctilio*, and winter moth.

To fulfill its mission, Otis Lab personnel maintain cooperative relationships with other Federal agencies, such as ARS and USDA's Forest Service, as well as State departments of agriculture, universities, and private industry. These cooperative arrangements also extend to government organizations and universities in a number of foreign locations, including Australia, Canada, China, Japan, Korea, New Zealand, Russia, and South Africa. The work includes developing methods to monitor and exclude AGM from North America, predicting the invasiveness of organisms by assessing damage on expatriate North American plants in foreign locations, developing and evaluating attractants, and developing control techniques for targeted exotic pests.

The CPHST California Station in Salinas also reports to the Otis Lab. This station focuses on LBAM, EGVM, and mass-rearing strategies for biocontrol agents of the ACP. The station develops control and detection methods for these pests and analyzes program data to help our stakeholders maintain export markets for affected commodities.

Recent Accomplishments

Pest Detection

- Identified attractants for over 100 species of Cerambycids as part of a wood borer attractant project funded by the Farm Bill. Specific information on attractants for Cerambycids and the exotic Buprestid, *Agrilus biguttatus*, was given to the CAPS program for incorporation in the CAPS wood borer survey.
- Prepared and distributed approximately 70,000 pheromone dispensers to participants in CAPS and national surveys.
- Successfully tested multi-species and multi-use traps in domestic locations on a number of target pest insects. Completed second year of pilot testing a risk-based model for EAB detection trapping with positive results.

Diagnostic Tools

- Developed 13 DNA markers to identify points of origin and pathways of intercepted gypsy moths. These markers have been added to the suite of markers used to screen specimens intercepted on ships and in port surveys. Many additional markers are now being tested to provide more precision and certainty about point of origin and flight ability of females.
- Examined with molecular diagnostics new ALB detections near existing infested areas to determine if these are the result of new introductions or natural spread. This information will help the ALB program to make appropriate operational decisions.
- In an ongoing port monitoring project, immature wood borers intercepted in wood packing material from six ports are being reared to the adult stage. The adults are identified and used to generate a DNA barcode for the intercepted species. DNA analysis can then be used to identify future interceptions of larval wood borers, which are usually unidentifiable by standard means.

Asian Longhorned Beetle

- Evaluated traps and new lures for ALB in Ohio and in China.
- Provided the ALB program with new recommendations on host tree susceptibility to ALB. A new host list was developed, and additional tree species are under investigation. Revisions in the ALB host list will have major impacts on the use of resources for survey and treatment.
- Completed a final report for PPQ about the long-term economic and ecological impact of ALB on the 19 Eastern States with hardwood forests. This report establishes an economic basis to justify the program's ALB eradication efforts.
- Investigated the utility of using detector dogs for ALB survey.
- Delivered a recommendation to the ALB program on the utility of using the current trap/lure combination for ALB survey.
- Produced a technical working group report on the Ohio ALB infestation with specific recommendations for the ALB program.
- Provided technical advice to the ALB program on the movement of wood from regulated areas and disposal of wood and wood waste. In the aftermath of Hurricane Sandy, this information allowed the ALB

program to safely move wood and wood waste out of the quarantine areas.

Emerald Ash Borer

- Developed a new trap design for the EAB and transferred this information to the program. Field test results in 2012 showed this trap to be superior to the current prism trap, and confirmatory tests were conducted in 2013.
- Tested the new EAB trap design in foreign locations, with preliminary results showing great promise for its utility in trapping other *Agilus* species of concern. We expect this new trap to become an integral part of the wood borer survey programs.
- Updated protocols for release and evaluation of parasitoid releases in the EAB biocontrol program, and provided a summary and evaluation of the previous releases that helped establish the future direction of the program.
- Completed nontarget host screening on a new imported Russian/Korean parasitoid, *Spathius galinae*, of EAB and applied for release permits. This species is better suited to colder northern areas of the United States and is expected to contribute significant mortality to EAB populations.
- Discovered two additional EAB parasite species in the Russian Far East and are in the process of bringing colonies to the CPHST Otis quarantine facility for nontarget testing.
- Developed methods to determine the establishment, spread, and parasitism levels of EAB parasitoids, including protocols for use of yellow pan traps and tree sampling. Also developed methods to assess ash stand health, including transect survey protocols for monitoring ash regrowth. These methods were developed in Michigan in cooperation with ARS, USDA's Forest Service, and Wayne State University and were transferred to cooperators in other States.

European Grapevine Moth

- Completed a technical working group report providing direction and methodology for the successful conclusion of the EGVM program.
- Delivered recommendations on traps that can be used when a mating disruption regimen for EGVM is in place.

Asian Gypsy Moth/Gypsy Moth

- Supported the AGM exclusion program by participating in bilateral meetings in Japan and Korea. Conducted

cooperative work in AGM source countries to define the range, population pressure, and behavior of AGM adults. These consultations and the technical input have resulted in a strengthened offshore inspection/certification program for AGM.

Sirex

- Transferred technology for the application and use of the parasitic nematode *Deladenus siricidicola* to the New Jersey Department of Agriculture for control of *Sirex noctilio* populations.

Phytosanitary Treatments

- Submitted technical information on radio frequency and microwave treatment to the International Plant Protection Convention (IPPC) for consideration as alternative treatments for wood-packing material. These were accepted in spring 2013. Previously, the only two treatments allowed under the ISPM-15 standard were methyl bromide fumigation and conventional heat treatment.
- Provided technical data to support a change in the ISPM-15 heat treatment standard and the heat treatment of domestic movement of wood to 60° C for 60 minutes to permit the safe movement of regulated wood and wood products.
- Offered technical advice to the EAB program on the movement of wood from infested area. This information helped allow regulated material to move from EAB quarantine areas.
- Developed effective new phytosanitary cold treatments for the Oriental fruit fly (*Bactrocera invadens*) and the peach fruit fly (*Bactrocera zonata*). These treatments will allow trade in fruit from areas known to be infested with these pests.
- Developed and delivered methyl bromide fumigation schedules for the fungal pathogen and walnut twig beetle involved in thousand cankers disease. This treatment has permitted movement of walnut wood from infested areas. We are also working toward development of a new treatment that does not use methyl bromide.
- Successfully tested vacuum steam treatments for use on veneer logs in shipping containers. Continuing work will focus on additional applications, including firewood and other commodities.
- Represented PPQ on the IPPC forestry panel, providing for changes in IPPC's ISPM-15 both in terms of new

treatments and on the allowable bark component on solid wood-packing material.

- Developed three space spray schedules for use in cargo holds and containers to disinfest for Japanese beetle and other flying hitchhikers using replacements for 10-percent d-phenothrin, which is no longer registered by the EPA.

California Station

- Developed a small-scale rearing system for EGVM capable of producing up to 10,000 larvae per week. This colony was used to test pesticides and develop improved tools for the EGVM areawide control program; the efficacy of new and existing materials and application rates were both tested. This information was delivered to growers through University of California Cooperative Extension.
- Cooperated with the University of California (UC) and ARS to develop fumigation schedules for table grapes using methyl bromide and other treatment alternatives. To support this work, a custom environmental control room for fumigation work for EGVM on table grapes was designed and installed inside a UC-Davis quarantine lab.
- Completed several projects with UC on EGVM mortality during wine-making procedures. The work showed that there is no risk of spreading EGVM by transporting red wine must, which allowed this commodity to be deregulated. The project results also demonstrated that regulatory treatment during the press process of 2 bars of pressure is effective, and that the grape crush process can allow significant numbers of larvae and pupae to survive if grape loads come from infested fields. These results highlight the need for increased sanitation at wineries that may be processing infested grapes.
- Supported the EGVM control program by conducting studies of phenology and surveys of alternate host plants in Napa County.
- Organized a working group with the strawberry industry, Monterey and Santa Cruz counties, LBAM Cooperative Program personnel, and UC to conduct a pest risk analysis of LBAM and develop a systems approach for conventional and organic strawberries that would establish streamlined regulatory procedures for interstate and foreign export of strawberries from quarantined areas.
- Worked with UC to test existing and new materials as regulatory treatments of LBAM on nursery stock. Results showed which materials were most effective to control different LBAM life stages and the duration of

effective control on field-grown material. Several treatments had extended control up to 3 weeks after treatment.

- Conducted a study on the use of irradiation as a phytosanitary treatment for LBAM using an X-ray source.
- Analyzed the horticultural mineral oils available in California and provided a recommendation to the LBAM program to allow the regulated industry more options for using these oils to control LBAM on nursery stock. This recommendation was adopted by CDFR, and growers now have more choices in their selection of approved oil treatments.
- Analyzed LBAM program data to estimate the spread rate of LBAM in California and prepared a written recommendation regarding a proposal to reduce the size of the quarantine area for new LBAM detections.
- Analyzed LBAM trapping records and scientific literature to provide a technical recommendation for the size of an effective quarantine area around a new LBAM find.
- Tested a new four-component pheromone lure under California environmental conditions and determined the use of the new lure would significantly improve trap catch over the current two-component lure.
- Initiated new work to mass rear *Tamarixia radiata* in California. This parasitoid is a biological control of the ACP, a vector for citrus greening.

CPHST Phoenix Laboratory, Phoenix, AZ

Laboratory Director: Richard Zink

The CPHST Phoenix Laboratory's mission is to develop, adapt, and implement areawide control technologies for new and existing program pests. Current work includes developing control tools, methods, equipment, and support for PBW and rangeland grasshopper/Mormon cricket. These control technologies include biocontrol, SIT, pheromones, new chemicals, and ground and aerial delivery systems. The lab's scientists conduct extensive laboratory and field developmental and operational scale studies to test and validate materials, methods, and equipment. The lab employs specialized equipment, including ground and aerial application technology; environmental chambers and mass-rearing modules; a twin-screw extruder for insect diet development; a room that allows for accurate simulation of aerial applications of sprayed products; a quarantine laboratory for rearing of genetically modified PBW; greenhouses; laboratory-located mini-rangeland and cotton field plots; and equipment for testing pesticide and pheromone application technology.

The Phoenix Lab's rangeland section works with Federal and State customers to provide technical assistance for the grasshopper and Mormon cricket control programs. This section also develops and implements solutions to program problems and continuously evaluates the technology and tools of the control program. The PBW section supports the PBW eradication program by providing expertise on pheromone mating disruption, custom rearing and mass rearing of insects, SIT mechanisms, insect population monitoring, and insect behavior. This section works closely with the PBW rearing facility, CPHST Albany facility, Arizona Cotton Research and Protection Council, ARS' Arid Lands Agricultural Research Center, University of Arizona's Entomology Department, local cotton growers, International PBW Eradication Program, and State and regional PPQ offices.

Recent Accomplishments

Rangeland Section

- Conducted a replicated trial that compared a candidate new grasshopper treatment, Rynaxypyr, to PPQ standard treatments: diflubenzuron, carbaryl, and malathion. Operational trials to determine the lowest reliable dose of Rynaxypyr were conducted in 2013.
- Conducted grasshopper control operational trials, replacing the crop oil component with an anti-drift deposition adjuvant in aerial applications of diflubenzuron. This will produce savings by simplifying the mixing and loading, while increasing aircraft use efficiency. The mix is ready for implementation.
- Continued work to find a new toxicant that will produce comparable rangeland grasshopper and Mormon cricket mortality to carbaryl in a bait formulation. Several commercially available, carbaryl-based baits have been field tested and found to be essentially equivalent. The concentrations tested of Permethrin- and Rynaxypyr-based baits were not as effective as others already available. Bifenthrin bait showed promise in cage tests.
- Collected, cataloged, and screened domestic pathogenic fungi from soil samples provided by PPQ personnel while surveying for grasshoppers. Developed and implemented screening protocol methods for ultraviolet and heat tolerance to spore and germination phases. Developed small, replicated plot methods to speed the search for suitable biopesticide field applications. New 'organic' carriers for the field application are also being developed.

- Conducted field exposure and aging studies of the exotic grasshopper-specific pathogen, *Metarhizium acridu*, which is used as a biopesticide for locust control in Africa and Asia. Application for a permit to study the pathogen under field conditions in mini-plots were carried forward in 2013.
- Continued to acquire, catalog, and make available field research data, historical documents, and other literature documenting grasshopper and Mormon cricket control efforts conducted by PPQ. The holdings include maps, photos, and meeting records that cover the development and history of rangeland pest management. The goal of this ongoing project is to provide access to documents, prevent the loss of relevant results, and build an information warehouse that will accommodate current and future information requests.

Pink Bollworm Section

- Tested and introduced strontium chloride as a second dietary marker of PBW released for SIT in the International PBW Eradication Program.
- Evaluated and recommended an increased sterilizing dose of radiation to the PBW Rearing Facility to reduce confusion in field traps from progeny.
- Reared, shipped, and tracked fresh, undyed, fluorescing PBW moths to program managers in four U.S. and two Mexican States for use as quality assurance in program traps.
- Produced and shipped PBW diet to at least eight U.S. and English cooperators for rearing diverse Lepidoptera species.

CPHST Plant Epidemiology and Risk Analysis Laboratory, Raleigh, NC

Laboratory Director: Robert Griffin

The CPHST Plant Epidemiology and Risk Analysis Laboratory (PERAL) is PPQ's primary unit producing pest risk analyses (PRAs). In this laboratory, a diverse group of scientists and professionals provides essential scientific support to risk-based policymaking across a broad range of phytosanitary issues. Staff members use sound science to analyze import, export, and operational issues. PRAs are essential to help safeguard American agriculture and plant health from harmful exotic plant pests in both managed and unmanaged ecosystems. More specifically, the analyses help

PPQ to design risk-based regulations for import and domestic pest management programs, identify and assess new pest threats, monitor the effectiveness of existing programs, and optimize available resources to enhance protection. PERAL personnel also provide the technical support documents PPQ requires for pests, commodities, and pathways. These products may include risk maps that indicate existing or potential range domestically or internationally or that predict ranges from weather- or climate-matching analyses.

PERAL has established itself as a global leader in both productivity and quality management. PERAL is currently the only International Organization for Standardization (ISO)-certified plant health risk analysis unit in the world. The group contributes significantly to the promotion of international dialogue and increased capacity for science-based management of phytosanitary issues through its Risk Analysis Mentoring Program for visiting scientists. In addition, PERAL provides basic PRA training workshops, with topics covering the spectrum of concepts, methods, and resources associated with PRA. Furthermore, PERAL promotes regional and international harmonization of plant health regulations by providing scientific support to PPQ in the North American Plant Protection Organization (NAPPO) and the IPPC.

Recent Accomplishments

- Finalized 21 original Q-56 risk assessments and pest lists in FY 2012. These risk assessments represent new potential imports of 45 commodities from 21 different countries. PERAL also completed one original Q-37 risk assessment and revised 31 Q-56 risk assessments and nine Q-37 risk assessments.
- Processed risk assessment and informational documents for the organism, pathway, citrus, and risk mapping areas, including six organism, eight pathway, and four ad hoc analyses. PERAL also completed 41 analyses in support of U.S. exports, involving 17 countries and 21 commodities.
- The New Pest Advisory Group (NPAG) completed 38 full NPAG reports and 52 pre-assessments for organisms that were not considered NPAG pests. Of the 38 pests that were the subject of full NPAG reports, 15 were deregulated after consultation with the National Plant Board, 19 received port policies of reportable/actionable, and two were sent to PPQ Plant Health Programs (Policy Management) for continued discussions with the States.

- The Deregulation Evaluation for Established Pests (DEEP) project analyzed 73 pests, 27 of which were determined to no longer meet the definition of a quarantine pest and were eventually deregulated at U.S. ports of entry after consultation with the National Plant Board.
- The PERAL Weed Team completed 29 original weed risk assessments and revised 6 weed risk assessments.
- Until its transfer to another unit in February 2012, the Exotic Pest Information Collection and Analysis (EPICA) team produced 14 weekly notifications containing approximately 50 articles on pests of regulatory significance.
- The Global Pest and Disease Database (GPDD) and Data Archival and Reporting Tool teams added about 800 pests to the database.
- Organized and delivered 6 risk analysis workshops (two of which were delivered in Spanish) and hosted scientists from 17 countries as participants in the Risk Analysis Mentoring Program.
- Provided support to PPQ headquarters on ongoing international trade issues, including a trade dispute with Mexico over potato exports and international standard-setting, by actively participating in the Harmonization Advisory Group, a new cross-functional team.

Biological Control Program, Raleigh, NC

Coordinator: Kenneth Bloem

The CPHST Biological Control Program is a virtual team of scientists (14–18, depending on project approvals from year to year) located at various CPHST locations. The program focuses on developing technologies that support the safe use of parasitoids, predators, herbivores, and pathogens to help mitigate the impacts of introduced invasive weeds and plant pests, while minimizing impacts on the environment and nontarget organisms.

CPHST scientists offer programs technical oversight and expertise to ensure that scientific knowledge gaps are identified and addressed, cooperators deliver needed services, and implementation protocols and educational materials are effectively developed and transferred to stakeholders as quickly as possible. More specifically, CPHST scientists provide permitted biocontrol agents collected from established field insectaries for distribution by PPQ and other project cooperators, develop new

rearing and monitoring systems, and work to ensure the safety of biocontrol agents by conducting both pre- and post-release impact studies. Project selection is based on national review recommendations from stakeholders within PPQ as well as the National Plant Board.

Recent Accomplishments

Canada Thistle

CPHST Fort Collins continued to assess the white rust pathogen *Pustula spinulosa* (formerly *Albugo tragopogonis*) from China as a potential biological control agent for Canada thistle. This pathogen has been reported as occurring in the United States on sunflower, and physiological “races” of this fungus have been reported. Further studies are needed to ascertain if the Chinese *Pustula spinulosa* is a different variety or race than the one known to occur in the United States. Field observations suggest that the Chinese *Pustula spinulosa* could be highly specific and damaging to this weed.

Diffuse and Spotted Knapweed

Cyphocleonus achetes is a weevil introduced into North America for the biological control of diffuse and spotted knapweed. Dispersal of *C. achetes* throughout the Western United States has been very slow due to low numbers of insects available for distribution. With a goal to facilitate the weevil’s redistribution, CPHST undertook a project to develop a rearing system based on artificial diet. The project was completed in 2012 after successfully rearing the weevil on artificial diet for 16 generations. With the help of cooperators at the Nez Perce Bio-Control Center, we were able to confirm that diet-reared insects from releases in 2010 and 2011 successfully established in the field, and the technology was transferred to the center.

Rush Skeleton Weed

In 2012, an Albany, CA, scientist initiated a project at the request of the Nez Perce Bio-Control Center in Idaho to develop an artificial diet for rearing *Brachyrrhoa gilveola* (pyralid moth), which is an agent for biological control of rush skeleton weed, *Chondrilla juncea*. Four different diets were tested: rice stem borer diet, pink bollworm diet, cactus moth diet, and the Hylobius diet developed at CPHST Albany. Our experiments showed that diet presentation plays a crucial role in feeding induction, with the highest establishment on diets dispensed with ridges. The highest and longest survival rates of *B. gilveola* were obtained on the Hylobius diet; larvae established, molted, and fed for 2 months.

Russian Knapweed

The Russian knapweed gall midge, *Jaapiella ivannikovi*, was permitted for U.S. release in 2009. A greenhouse-based rearing program for *J. ivannikovi* was initiated at Fort Collins in 2011 and continued in 2012. Rearing data were collected to document the productivity of the program and suggest ways to improve its efficacy. In 2012, nearly 1,600 *J. ivannikovi* galls were provided for field release at 38 sites in 8 States: California, Colorado, Idaho, New Mexico, Oregon, Utah, Washington, and Wyoming. The 2012 Utah releases represent the initial *J. ivannikovi* introductions in that State. Postrelease galling was observed at a few sites initiated in 2011, suggesting successful establishment. To date, project partners have released *J. ivannikovi* at 57 sites in the eight States mentioned above.

In 2012, we also conducted a preliminary greenhouse study quantifying individual and combined impacts of a biocontrol agent (*J. ivannikovi*) and a competitive perennial grass (sideoats grama) on Russian knapweed plants. Grass competition reduced some above-ground growth parameters while the gall midge did not; treatment interactions were not significant. We expanded this study in 2013, utilizing field cages and increased replication.

Yellow Toadflax

In 2012, CPHST Fort Collins successfully continued a greenhouse-based rearing program (begun in 2011) for the yellow toadflax stem-mining weevil, *Mecinus janthinus*. Much of the 2012 effort concentrated on developing a better understanding of adult eclosion biology, which will be used to optimize rearing efforts. It appears that adult weevils can be produced from early spring through late summer by adjusting the duration of cold storage, although longer cold treatment may be accompanied by increased adult mortality. We did not make field releases with reared weevils in 2012; we used all weevils collected to produce more weevils for 2013 field releases. However, PPQ partners in Montana provided field-collected *M. janthinus* for release in Colorado, Idaho, North Dakota, Oregon, South Dakota, Washington, and Wyoming in 2012.

Asian Citrus Psyllid

From September 2011 through September 2012, over 120,000 *Tamarixia radiata* parasitoids were mass-produced and released by the CPHST Mission Laboratory in the Lower Rio Grande Valley (LRGV) of Texas for ACP management. Of these, over 35,000 were released within a 5-mile radius of where citrus greening disease was

detected in January 2012. Both open and closed releases continue and are being used to assess establishment and efficacy of the parasitoids. Open field releases have been made at 135 release sites at an average of 923 parasitoids per site. Releases in the urban environment are being made in collaboration with Master Gardeners, Texas A&M Agrilife Extension, and Texas Citrus Mutual using field insectary cages in recreational vehicle (RV) parks to help augment parasitoid release numbers. Recovery of parasitoids has been confirmed at 39 locations (17 inside the HLB-quarantine zone and 22 outside). Closed cage releases made in fine-mesh sleeve cages indicate average parasitism levels of 10.4 percent. Host feeding studies indicate additional mortality at 64.9 percent. Using the field cage-rearing methods developed by the Mission Laboratory, the CPHST California Station initiated a cooperative effort with the Citrus Research Board, CDFA, and UC to develop field cage mass-rearing techniques for *T. radiata* in California.

Emerald Ash Borer

Methods to determine the establishment, spread, and parasitism levels of EAB parasitoids were developed in Michigan in cooperation with ARS, USDA's Forest Service, and Wayne State University. These methods included protocols for use of yellow pan traps and tree sampling, as well as methods to assess ash stand health (such as transect survey protocols for monitoring ash regrowth). These methods are now being shared with and transferred to cooperators in other States, including New York and Tennessee. CPHST Otis, in collaboration with ARS in Newark and Delaware, also conducted host specificity tests for a new *Spathius* species from Russia. A permit application for field release was recently submitted. The new species from Russia may be more cold tolerant than *S. agrili* from China, which was collected from a considerably warmer climate.

Grasshoppers

CPHST Phoenix, in cooperation with ARS, finished early season exposure persistence tests with the fungal pathogen *Metarhizium acridum* for grasshopper control. PPQ will use the data to respond to public comments on an environmental assessment for the release of commercial Australian and African strains for small plot tests. Trials have indicated that the pathogen persists, but remains below levels that would be expected to produce infections in native populations. If APHIS issues a finding of no significant impact based on the data collected, CPHST will conduct small field trials as soon

as practical. The exotic fungi will be compared with native pathogens with ultraviolet light and heat tolerance.

Harrisia Cactus Mealybug

Harrisia cactus mealybug (HCM, *Hypogeococcus pungens*) is a severe pest of columnar cacti worldwide and a major threat to endangered endemic cacti in Puerto Rico and the ornamental industry in the mainland United States. Working with cooperators in Puerto Rico, the CPHST Mission Lab identified two endemic parasitoids that attack HCM. Genetic comparison of the most prevalent parasitoid species, *Leptomastidea* sp., indicates that it is identical to populations attacking HCM in Barbados and Florida. Species-level identifications are pending taxonomic review by an Encyrtid specialist at UC-Riverside.

Red Bay Ambrosia Beetle

The red bay ambrosia beetle, *Xyleborus glabratus*, is a vector of laurel wilt disease that has caused widespread and almost complete mortality of bay and avocado trees in infested areas. CPHST Miami collaborated with University of Florida scientists to develop a method using sentinel logs infested with the beetle to collect potential natural enemies attacking them. Multiple families from the order Hymenoptera (Scelionidae, Braconidae and Eulophidae) emerged from trees infested with *X. glabratus* and other Scolytinae. According to current scientific literature, species in these families have been identified as parasitoids of Scolytinae, and efforts are now underway to confirm host associations.

Sirex Woodwasp

FY 2012 was the final year of CPHST's *Sirex* biological control project using Australian nematodes. The last controlled releases of the imported Kamona strain of *Beddingia siricidicola* were made in September 2012 in four red pine plantations in Pennsylvania. Releases in the previous season (2011/2012) compared infection rates when girdled trap trees were inoculated at three times in the season. Altogether, the Otis Lab undertook controlled releases over seven seasons in this project and collected DNA samples from infected wasps over 5 years. The latter samples are being analyzed to discriminate the Kamona and "native" North American strains. When complete, these analyses will provide a good picture of the relative effectiveness of the two nematodes. We also investigated hybridization of the strains, which was observed in the lab and, in the long run, may impact the utility of the imported biological control agent in the field.

Tomato Leafminer

Tuta absoluta is a devastating pest of tomato. Miami bio-control scientists determined that local nontarget gelechiid moths in Miami-Dade County are attracted to the two-component lure used by CAPS. CPHST designed and analyzed experiments conducted in Panama and Miami that determined a new adhesive insert was as effective in trapping *T. absoluta* as standard traps while allowing for faster visual processing. CAPS used the results to modify trapping protocols used in the United States. Efforts are currently underway, in cooperation with researchers from Instituto Valenciano de Investigaciones Agrarias in Valencia, Spain, to assess the local native enemy community in Florida and test for their potential to control *T. absoluta*. In Spain, integrated pest management practices using generalist natural enemies now largely control the pest.

Imported Fire Ant

Since 2002, two to three species of *Pseudacteon* sp. flies have been released at multiple sites in all IFA-quarantined States in the contiguous southeastern States and Puerto Rico. Field releases with a fourth species, *P. cultellatus*, began in 2011. From 2002 through 2012, there have been 138 field releases in IFA-quarantined States in the contiguous southeastern States and Puerto Rico (no releases in New Mexico and only one species released in California), and more than 1.5 million potential flies have been released or used in demonstration/research projects. Of these 138 releases, 67 were *P. tricuspsis*, 45 were *P. curvatus*, 23 were *P. obtusus*, and 3 were *P. cultellatus*. Through APHIS releases, along with other Federal and university releases, *P. tricuspsis* is well established in the southern areas of the IFA-regulated area—covering over 50 percent of that area. To date, *P. tricuspsis* is not known to be established in California, Oklahoma, or Tennessee. The second species, *P. curvatus*, is well established in all southern IFA-regulated States and Puerto Rico, covering about 65 percent of the regulated area. *P. curvatus* has not been released in California. Overwinter establishment of *P. obtusus* has been confirmed with very limited expansion at this time, and overwintering of *P. cultellatus* has not yet been confirmed.

Fruit Fly Program, Raleigh, NC

Coordinator: Pat Gomes

The CPHST Fruit Fly Program supports APHIS Fruit Fly Exclusion and Detection Programs in meeting goals to: strengthen detection and response capabilities, conduct

preventative sterile fly releases over areas susceptible to fruit fly outbreaks, and carry out ongoing control programs to prevent exotic fruit flies from becoming established or spreading within the United States.

In 2012, Pat Gomes assumed responsibilities as the CPHST Fruit Fly Program Coordinator, and John Stewart became the National Policy Manager for Fruit Fly. Program efforts centered on providing continued technical and methods support to the field during emergency response quarantine and eradication activities. These include support to improve fruit fly rearing and emergence techniques; fruit fly aerial sterile releases; release equipment calibration; ground pesticide treatments and operational techniques; enhanced detection; modeling of pest colonization and dispersal; and quality assurance of program resources such as lures, diets, and control pesticides.

CPHST provided important technical and scientific support to the control and eradication efforts that took place during 2012. In California, two quarantines were imposed for oriental fruit fly in Stockton and Anaheim, while a single Medfly outbreak near Rancho Cucamonga required regulatory action. Although PPQ and the Texas Department of Agriculture announced eradication of the Mexfly from Texas in January, outbreaks occurred across the entire LRGV in March. In 2012, Florida was entirely free of fruit fly detections and outbreaks.

Recent Accomplishments

- Calibrated irradiators used to sterilize Mexfly and Medfly using alanine transfer dosimeters for certification by the National Institute for Standards and Technology.
- Approved the use of a new, more cost-effective solid cone formulation of three-component lures to replace standard three-component patches for use in APHIS cooperative fruit fly programs.
- Delivered sterile Mexfly black pupal strain for program use in Texas.
- Delivered a new strain of Mexfly (Willacy standard strain) for production, sterilization, and release in Texas.
- Convened a technical working group to review the Mexfly program in Texas and formulated recommendations to improved rearing, SIT, and eradication efforts.
- Through the Mexfly program in Texas and Mexico, developed and implemented interpolated maps using sterile fly capture data to evaluate sterile fly densities within release blocks on a biweekly basis.
- Validated sanitation methods for bacterial pathogens (two species of *Morganella*) that affect mass rearing of Mexfly and delivered these methods to the Edinburg rearing facility for program use.
- Calibrated Mexfly aerial release machines to release a more accurate number of fruit flies per acre.
- Performed a quality assurance review of the Mexfly Rearing and Emergence Facility in Edinburg, TX, and monitored environmental conditions within the facility.
- Successfully tested an oil of wintergreen mixed with malathion to mask the unpleasant odor during treatments of production areas for Oriental fruit fly and spotted-winged *Drosophila* in California. This scent is expected to reduce complaints by homeowners living near treated fields.
- Improved identification techniques and tools.
- Developed a Medfly host compendium and updated host lists for regulatory quarantine.
- Produced a report on fruit fly host status titled “Host plants of *Bactrocera dorsalis* complex belonging to family Cucurbitaceae.”
- Validated additional molecular methods to support pathway analysis, regulatory decisions, and identification for Oriental fruit fly and species of *Anastrepha*.
- Refined an SIT monitoring model for sterile release evaluations and regulatory decision-making.
- Supported additional pesticide labels for preharvest treatments in California for Oriental fruit fly and spotted-winged *Drosophila*.
- Convened a Systems Approach Working Group to address quarantine movement of major commodities, including fresh tomatoes from areas quarantined for Medfly, sweet cherries from areas quarantined for Oriental fruit fly, and citrus from areas quarantined for Mexfly in Texas.
- Developed additional pesticide quality assurance bioassays.
- Conducted bioassays and chemical analysis of fruit fly attractants in support of Field Operations as part of the CPHST Quality Assurance Survey Program.
- Evaluated aerial swaths used during preventative releases of sterile fruit flies in Florida and California to determine effective dispersal patterns and possibly reduce program costs.

- Provided fruit fly rearing and diet microbial contamination analysis and recommended new procedures to prevent contamination.
- Developed new, more cost-effective packaging and shipping methods for sterile fruit fly pupae.

National Plant Protection Laboratory Accreditation Program, Raleigh, NC

Coordinator: Patrick Shiel

The NPPLAP evaluates laboratories to ensure their capability in making accurate molecular diagnostic determinations for regulatory purposes. In addition to ensuring lab capability within PPQ and in other USDA agencies, NPPLAP engages the NPDN and State laboratories to increase capacity and proficiency in diagnostic testing among a dispersed laboratory network. NPPLAP fosters the adoption of practices that promote continuous improvement and accreditation standards. NPPLAP works extensively with the NPDN to develop and deploy a functional Quality Management system through partnerships with established accreditation bodies. This is accomplished through several Farm Bill projects and extensive interactions with the National Animal Health Laboratory Network and the American Association of Veterinary Laboratory Diagnosticians.

The goals of this program are to establish a state of readiness when needed by PPQ in emergency situations and to enable plant disease testing labs to provide diagnostic determinations recognized by USDA regulatory programs. NPPLAP currently accredits laboratories for USDA regulatory molecular diagnostics and provides yearly certifications to diagnose *P. ramorum*, *Plum pox virus*, and the HLB (citrus greening) pathogen. This is accomplished by conducting NPPLAP lab inspections for accreditation of new labs and certifying analysts through a yearly proficiency test program deployed by the CPHST Beltsville Lab Proficiency Test Group.

Recent Accomplishments

- Approved 17 labs and 27 analysts for *P. ramorum* testing. There was a 16-percent increase in proficiency test participation over 2010.
- Approved 12 labs and 35 analysts for HLB testing. Proficiency test participation increased by 21 percent, and participant submission of results decreased in time to an average of 14 days.

- Approved 13 analysts using a new proficiency test for ELISA screening of *Plum pox virus*. The analysts were from 9 separate labs nationwide.
- Analyzed testing data to track trends and inform proficiency test group decisions.
- Organized training and provided technical support to promote a CPHST-NPDN quality management initiative in plant diagnostics. Conducted three practice audits as part of this initiative to develop and deploy quality management system components, including auditor training and document development.
- Participated in the DHS Integrated Consortium of Laboratory Networks.
- Developed a Web portal for administering NPPLAP proficiency tests. This updated approach has helped to streamline deployment, review, and notification of proficiency test programs for diagnosticians and further ensure confidentiality and record integrity.

National Scientific Technologies Program, Riverdale, MD

Coordinator: Laurene Levy

CPHST's National Scientific Technologies Program (NSTP) is a new program established by PPQ Science and Technology in 2012 to assess and transfer detection, identification, diagnostic, and exclusion tools and technologies to support PPQ programs. The NSTP will ensure that PPQ uses scientifically sound tools and technologies with performance characteristics that not only enhance PPQ responses to emergency and domestic pests, but also strengthen pest exclusion and prevention activities at plant inspection stations and ports of entry. The NSTP scans scientific resources for existing and new, innovative tools and technologies for potential adaptation for PPQ programs. Further evaluation and validation is coordinated by the NSTP within PPQ or through cooperation with scientists at government, academic, and commercial industry labs. Selected tools and technologies are then implemented through training and pilot studies coordinated with PPQ leadership and programs.

The NSTP's current initiative is to strengthen strategic partnerships and facilitate dialogue among the PPQ Science and Technology, Policy Management, and Field Operations core functional areas to determine needs and logistics. NSTP also works to develop strategic partnerships with researchers and operational personnel

throughout USDA and other Government agencies, such as the U.S. Departments of Defense, Justice, Energy, and Homeland Security (especially DHS' Science and Technology Directorate [DHS S&T] and Customs and Border Protection [CBP]); domestic and international academic institutions; commercial industry; and national plant protection organizations.

Recent Accomplishments

- Partnered with DHS S&T to design an optimized national plant pest detection system that will benefit PPQ and CBP. Working with DHS S&T and their contractor, Gryphon Scientific, the NSTP coordinated interview sessions with PPQ and CBP staff to understand the requirements and needs of the end users and other stakeholders. This information will be used to recommend specific technologies suited to a plant pest detection system.
- With representatives from PPQ S&T, Policy Management, and Field Operations, participated in the Molecular Diagnostics Task Force. The group is considering the potential for using molecular technologies in PPQ.
- Continued participation in the DHS Integrated Consortium of Laboratory Networks Methods and Logistics subgroups.

Project Highlights

CPHST AQI Lab Developments: Renovation of Building 63 in Miami, Closure of the Gulfport Lab, and Opening of the Biloxi Station

Location: CPHST AQI Lab and Biloxi Station

Lead Scientists: Woodward Bailey, Anne-Marie Callcott, and Michael Hennessey

Team Members: Timothy Bond, William Guyton, Clinton Harley, Richard King, Lisa Mosser, Amy Roda, Robert Smith, Kayimbi Tubajika, Xikui Wei, and Scott Weihman

In 2012, many advances were made toward actualization of the CPHST AQI Lab. The CPHST AQI Lab is based in Building 63 of the USDA Agricultural Research Service (ARS) Subtropical Horticulture Research Station in Miami, FL. The lab also has staff in Raleigh, NC (formerly known as the Treatment Quality Assurance Unit) and Biloxi, MS (formerly known as the CPHST Gulfport Lab).

The CPHST Gulfport Lab officially closed on September 7, 2012; all Gulfport staff and activities were redirected and relocated. All carryover field and laboratory operations were completed from February to April 2012, and all activities transferred to the CPHST Miami Laboratory or outsourced. The primary PPQ mission-related activities that remain with the Biloxi staff include overseeing the pesticide residue analysis program for environmental monitoring samples associated with PPQ treatment programs and the development of imported fire ant (IFA) regulatory/quarantine treatments to support the Federal IFA Quarantine and the rearing and release of phorid flies (biocontrol agents of IFA). The Miami renovation and Biloxi relocation represent an immense undertaking for all involved CPHST staff members. Their contributions and sacrifices are greatly appreciated by the CPHST management.

Gulfport Closing/Biloxi Opening Highlights

- Worked with Mississippi State University (MSU) to establish a lease agreement and cooperative agreement for office space at the Biloxi site.
- Worked with PPQ information technology (IT) staff, MSU staff, and contractors to wire Biloxi offices for phones.
- Worked with employees at the Gulfport facility to assist in their relocation as needed.
- Managed excess property at the Gulfport facility. Over 90 percent of inventoried property was transferred or remained on site, and over 80 percent of non-inventoried property was transferred or placed by the U.S. Government Services Administration

(GSA) for sale; remaining property was listed for sale with GSA in 2013.

- Shipped an 18-wheeler with transfer property to the Mission Lab, as well as an 18-wheeler and a smaller truck with transfer property to Miami.
- In August 2012, completed final safety review/audit required for closure of the Gulfport facility and removed all hazardous waste from the premises.
- Throughout the year, cancelled or modified contracts to provide as much savings as possible.
- APHIS Environmental Services must complete soil remediation prior to GSA accepting property for disposal. The facility technician will remain on the Gulfport property until transfer to GSA to provide protection and maintenance as required.

Building 63 Renovation Highlights

The \$1.1 million renovation of Building 63 in Miami, FL, began in 2011 and was completed in 2012. This facility is now a state-of-the-art showcase for CPHST AQI activities. The main highlights of this project are listed below.

- Four new lab areas added to floor plan
- Modified heating, ventilation, and air conditioning systems
- New electrical system, including new power distribution feed
- New ceilings and floors
- Strengthened roof
- New systems furniture (cubicle workspace)
- New interior doors
- New interior and exterior paint
- New laboratory equipment, including incubator and environmental growth chambers

A Novel Virus, of the Genus Cilevirus, Causing Symptoms Similar to Citrus Leprosis

Location: CPHST Beltsville Lab

Lead Scientist: Mark Nakhla

Team Members: Gang Wei and Deric Picton

Cooperators: Avijit Roy, Nandlal Choudhary, Leon M. Guillermo, Jonathan Shao, Ananthakrishnan Govindarajulu, Diann Achor, John S. Hartung, Laurene Levy, and R.H. Bransky

Citrus leprosis virus (CiLV) was originally described from Florida over 100 years ago and later found in Argentina, Brazil, Paraguay, and Uruguay where it is endemic. Recently, the disease was detected in several additional countries in Central and South America. The proximity of these detec-

tions and potential damage the virus can cause are a concern in the United States, where the disease has not been observed since the 1960s. CiLV produces chlorotic lesions with or without necrotic centers on citrus leaves, fruit, and twigs. Sweet orange and mandarins are considered to be the most affected citrus species. The virus is transmitted by the false spider mite *Brevipalpus* spp. There are two known types of CiLV: the virus particles of the most prevalent “cytoplasmic type,” are found mainly in the cytoplasm (CiLV-C), and virus particles of the nuclear type reside primarily in the nucleus of infected cells. Only CiLV-C is molecularly characterized, and it belongs to a novel virus genus, *Cilevirus*.

Citrus leprosis in Colombia was previously shown to be caused by CiLV-C. In 2011, enzyme-linked immunosorbent assay (ELISA) and reverse-transcription polymerase chain reaction (RT-PCR)-based diagnostic methods failed to identify CiLV-C from citrus samples with symptoms similar to citrus leprosis. However, virions similar to CiLV-C were observed in the cytoplasm of the symptomatic leaves by transmission electron microscopy. Furthermore, the causal organism was transmitted by the false spider mite, *Brevipalpus phoenicis*, to healthy citrus seedlings.

A library of small ribonucleic acids (RNAs) was constructed from symptomatic leaves and used as the template for high-throughput parallel sequencing. The complete genome sequence and structure of a new bipartite RNA virus was determined. RNA1 (8,717 nucleotides) contained two open reading frames (ORF). ORF1 encoded the replication module, consisting of five domains: methyltransferase (MTR), cysteine protease-like, FtsJ-MTR, helicase, and RNA-dependent RNA polymerase. ORF2 encoded the putative coat protein. RNA2 (4,989 nucleotides) contained five ORFs that encode the movement protein and four hypothetical proteins. The structure of this virus genome resembled that of CiLV-C, except that it contained a long untranslated terminal region and an extra ORF in RNA2. Both the RNA1 and RNA2 of the new virus had only 58- and 50-percent nucleotide identities, respectively, with known CiLV-C sequences; thus, it appears to be a novel virus infecting citrus. Phylogenetic analyses of the ORF1 domains also indicated that the new virus was closely related to CiLV-C.

We suggest that the virus be called *Citrus leprosis virus* cytoplasmic type 2 (CiLV-C2), and it should be unambiguously classified as a definitive member of the genus *Cilevirus*. We designed and validated a pair of CiLV-C2

genome-specific RT-PCR primers to detect its presence in citrus leprosis samples collected from the Casanare and Meta states in Colombia.

CPHST CAPS Support: Bringing Pests, Surveys, Plants, and Science Together

Location: CPHST Fort Collins Laboratory

Lead Scientists: Lisa Jackson (arthropods and mollusks) and Melinda Sullivan (pathogens, nematodes, and weeds)

Team Members: Talitha Molet and Christina Southwick

The Cooperative Agricultural Pest Survey (CAPS) conducts science-based national and State surveys that target specific exotic plant pests, diseases, and weeds identified as economic and environmental threats to U.S. agriculture and/or the environment. USDA is the primary funding source for CAPS activities, giving funds to State departments of agriculture, universities, and other entities through cooperative agreements. The CPHST CAPS Support Team (CCST) provides the scientific foundation, develops products and tools, and offers day-to-day support for the CAPS program.

Commodity-Based and Taxon-Based Survey Manuals

The CCST has developed a series of survey references and guidelines for CAPS cooperators to assist with commodity-based surveys in corn, grape, oak, pine, small grains, soybean, and stone fruit and taxon-based surveys for exotic wood borers and bark beetles. In 2012, the team completed the “Cotton Commodity-Based Survey Reference and Guideline” and the “Asian Defoliator Pathway-Based National Survey Reference.”

Pest Datasheets

The CCST worked in 2012 to develop stand-alone datasheets, similar to those provided in the commodity-based reference documents, for eight insect, nine plant pathogen, and four nematode pests. Datasheets were also revised for seven insects and one mollusk pest.

CAPS-Approved Survey and Diagnostic Methods

The CCST previously developed a suite of tables for the CAPS program that provides the CAPS-approved survey and diagnostic method for each of the CAPS target pests beginning in 2010. To enter survey data, the CAPS community must use the CAPS-approved methods for survey and identification. CPHST continues to develop approved methods for new CAPS target pests. The approved methods information

is available on the CAPS Resource and Collaboration Web site at http://caps.ceris.purdue.edu/approved_methods.

Tuta absoluta Trap Efficacy Project

The CCST co-lead a project with scientists in the CPHST Miami Lab and PPQ Florida to evaluate traps for *Tuta absoluta*, the tomato leaf miner. The previous trap and lure combination captured many nontargets, which increased the difficulty and time needed to identify the specimens. As a result of field studies in Panama and Florida and work with a PPQ Domestic Identifier, the CAPS program now recommends using new trap inserts in its *Tuta absoluta* surveys. This successfully illustrated how the CCST identified a need, quickly conducted appropriate studies, and supplied a practical recommendation to the CAPS leadership.

Survey Protocols

In 2012, the CCST cowrote the *Cerceris* Wasp Survey Protocol. *Cerceris* wasps are an effective survey tool that can be used to detect new populations of exotic buprestids, including several CAPS targets. The CCST team also worked with California (PPQ and the California Department of Food and Agriculture [CDFA]) to develop a visual survey protocol for early detection of kiwi canker.

Pest List Review

The purpose of the Pest List Review was to review the process behind the Analytical Hierarchy Process (AHP), which is used to develop the Prioritized Pest List for the CAPS program. The CCST developed two assessments in this process. The preassessment questionnaire is used to assess new CAPS pest suggestions before they are run through the AHP model. The postassessment questionnaire evaluates the survey and diagnostic methods of pests that make it through the preassessment and the AHP model.

This new process was implemented in 2012. Out of 29 suggestions from the CAPS community, 19 passed the preassessment process and were run through the model. The group will run any new pests through the postassessment questionnaire to determine if they are appropriate survey targets for the 2014 survey season.

The AHP model is currently being revised by CPHST Plant Epidemiology and Risk Analysis Laboratory (PERAL) staff with the assistance of CCST. To date, the group has developed a set of draft questions. Once evaluated, the resulting questions will better predict the impact of new exotic pests

based on the biology, pest status in its current distribution, host presence, and the climatic suitability in the United States for the pest. These new questions will be used to develop the 2016 Prioritized Pest List.

Outreach

In 2012, CCST was asked to develop outreach documents for *Tuta absoluta* and *Neoleucinodes elegantalis*. The CCST worked with PPQ staff and APHIS Legislative and Public Affairs to create the CAPS Pest Profile, a new outreach product for imminent pest threats that is targeted for general public and private industry audiences. The CCST also developed three Pest Cards for *Adoxophyes orana*, *Eupoecilia ambiguella*, and *Spodoptera littoralis* to be used as outreach tools at the 2013 Southeast Regional Fruit and Vegetable Conference. Pest Cards are available at www.hungrypests.com/partner-tools.

New Pathogen Diagnostic Methodologies for Phytoplasmas and Viroids

Two Farm Bill-funded projects were initiated by the CCST to support CAPS sample pathogen sample processing and new methods development/validation for phytoplasmas and viroids in collaboration with Nigel Harrison (University of Florida), Robert Davis (USDA-ARS), and Rosemarie Hammond (USDA-ARS).

Support for CAPS Field Staff

The CCST serves as an informal “help desk” for the CAPS field staff. In 2012, Fort Collins scientists received 47 insect information requests from CAPS Pest Survey Specialists and State Survey Coordinators. The CCST also provided support to CAPS field staff on a range of pathogen issues, including clarifying survey and diagnostic methods for a wide variety of plant pathogens.

Survey of *Brevipalpus* Mites and Citrus Leprosis Virus in the Lower Rio Grande Valley of Texas and on Limes Imported From Mexico

Location: CPHST Mission Lab

Team Members: Roxanne E. Farris, Evan Braswell, Josie Salinas, Bacilio Salas, Danny Vasquez, Danny Martinez, Armando Loya, Terrance Todd, Laura Torres, Mark Nakla, and Laurene Levy

CiLV causes devastating economic losses to the citrus industry. The disease nearly destroyed the Florida citrus industry around 1925, but dropped to undetectable limits by the early 1950s.

Recognized in Argentina in the 1930s, CiLV has since been moving northward through South and Central America. The recent discovery of this virus in several states of southern Mexico has created concern that the disease could eventually reach the United States. This fear is exacerbated by the widespread distribution of the disease vectors in citrus-producing areas. The virus is transmitted by flat mites, also known as false spider mites, in the genus *Brevipalpus*. Traditionally, *B. phoenicis* was recognized as the vector of citrus leprosis in Brazil, *B. obovatus* in Argentina and Venezuela, and *B. californicus* in Florida. However, recent results suggest each of these classifications represents a unique species group.

Surveys of mites on citrus in the Lower Rio Grande Valley of Texas revealed that each of these species groups is present. This greatly increases the possibility that CiLV would become readily established if introduced there. This area is also the site for the vast majority of citrus importation from Mexico, and is the closest point in the United States to CiLV-infested areas. Once they acquire the virus, flat mites can transmit CiLV throughout their lifetime, even if they are maintained on nonsusceptible plants.

Due to the threat of CiLV entering the United States, it became important to evaluate shipments of Mexican limes entering the country. To facilitate this effort, we worked closely with CBP at the Pharr, TX, port of entry on sampling Persian and key limes from Mexico. Washing methods were developed to detect *Brevipalpus* mites on the limes using a pressurized hot water treatment. Laboratory tests showed 86-percent efficacy in removing mites from the citrus fruit. Live mites of *B. phoenicis* were intercepted on Persian limes coming from Veracruz and Queretaro, Mexico, even though the limes had undergone a wash and wax treatment at the packinghouses prior to entering the United States. Both Veracruz and Queretaro have been confirmed for the presence of CiLV.

Since morphological taxonomy of these mite species has failed in the past to recognize many species, we are developing molecular diagnostic tools such as real-time PCR to allow quick and accurate identifications. We are collaborating with morphological taxonomists to obtain *Brevipalpus* samples that are expertly identified to ensure the integrity of the molecular tools we develop. Determining the species that are able to vector CiLV is key to assessing the risk of spread; therefore, we are developing ways to detect the virus from the mites.

CiLV is an RNA virus; thus, it was necessary to evaluate extraction procedures that included RNA. We have demonstrated the ability to extract viral RNA and amplify the virus from a single mite specimen. Subsequently, we demonstrated the ability to jointly extract DNA and RNA from a single mite. This is of great value because the DNA will provide us with a means to identify the mite species, while the RNA will allow us to determine whether or not the virus is present. Currently, we are testing DNA and RNA extraction methods to determine the optimum method of nucleic acid isolation from a single mite specimen. We are also evaluating collection and storage conditions to determine which handling procedures will result in the optimum method for genetic analysis. This work will identify which mite species are prevalent in the Lower Rio Grande Valley and which species vector CiLV—information critical to a response if an infestation were to occur.

Development of a Global Gypsy Moth Microsatellite Database

Location: CPHST Otis Laboratory

Lead Scientist: Vic Mastro

Team Members: John Molongoski, Deborah Winograd, Artemis Louyakis, Yunke Wu, and Dave Lance

Cooperators: Richard Harrison and Steve Bogdanowicz
(Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY)

There are three recognized subspecies of gypsy moth, *Lymantria dispar*: European, Asian, and Japanese (Pogue & Schaefer 2007). European gypsy moths (EGM) became established in the northeastern United States more than a century ago, but the two Asian subspecies (collectively called Asian gypsy moths, or AGM) do not occur here. Female AGM (but not EGM) are capable of sustained flight, and AGM larvae will feed on a relatively wider range of plant species, especially in the early instars. Thus, APHIS-PPQ considers AGM to be a separate and potentially more damaging pest than EGM and regulates AGM as if it were a separate species. Also, according to Pogue and Schaefer's new classification several other forms of gypsy moth have been separated out as new species (*Lymantria albescentis*, *L. umbrosa*, and *L. postalba*). These, from a regulatory stand point, are treated as AGM. Several other species in this genus are also regulated and some are equally hard to distinguish in immature stages. The threat is not abating—indeed, the number of viable AGM egg masses intercepted at coastal ports in the continental United States has increased dramatically over the past several years.

Subspecies of *L. dispar*, in any life stage, cannot be determined reliably based on morphology. A number of years ago, three molecular markers were developed and have been useful for distinguishing whether many of the gypsy moths intercepted at ports or captured in traps were AGM or EGM. Unfortunately, those markers cannot unequivocally assign all specimens to EGM or AGM, and they provide minimal information on the geographic source of the population. We have identified and are currently evaluating a number of microsatellite markers in gypsy moths that may increase our ability to predict their source population or geographic origin. Microsatellites are tandemly-repeated DNA sequences having repeat unit lengths of 2–5 base pairs (for example, [GTT], [AT] or similar). Their total length varies due to increases or decreases in the number of repeats found in different gypsy moth populations. In order for microsatellite markers to be of maximum utility in determining source populations, we have constructed a microsatellite database or library for gypsy moths worldwide. Gypsy moths from across Europe, Asia, and four U.S. States (New Jersey, Wisconsin, Michigan, and Connecticut) have been screened with 9 and, in some cases, 13 microsatellite markers. To date, approximately 1,800 gypsy moths distributed worldwide have been examined by the team.

Data generated using multiple microsatellite markers will allow us to determine multilocus genotypes for individual gypsy moths and to potentially assign the intercepted or captured specimens to a foreign point of origin. We are analyzing the microsatellite data we have obtained from our global gypsy moth collection using the software STRUCTURE. This software employs a model-based clustering method for inferring population structure through the use of genotype data (Pritchard et. al., 2000). The software allows investigators to determine population structure, identify distinct genetic populations, and assign individuals to populations. The populations examined to date fall into 15 major and minor clusters representing four major geographic populations. Moving from West to East, the European population represented by France, Germany, Italy, and Hungary is distinct from the Central Asian population (Kazakhstan and Kyrgyzstan), which in turn is quite different from the population in eastern Asia (China, South Korea, Far East Russia, and Japan). Finally, the continental U.S. gypsy moth population is genetically quite different from the remainder of the world population. STRUCTURE analysis was also applied to a group of gypsy moth egg masses intercepted in Long Beach, CA, and in New Orleans, LA, respectively, in 2011. These data suggest that the California intercepts likely originated in Japan, while those intercepted in

Louisiana may have their origin in China, South Korea, or Far East Russia.

Future endeavors with this project will include: expanding the global gypsy moth microsatellite database to add more countries and geographic locations and testing additional recently discovered microsatellite markers to better refine our ability to genetically distinguish gypsy moths originating from each of the four primary eastern Asian countries. In addition, we will more closely examine domestic U.S. moth populations with these microsatellite markers to detect any evidence of introgression of AGM genes into domestic populations. Finally, we hope to optimize PCR multiplexing of chosen subsets of the microsatellite loci to substantially reduce the time, effort, and expense required to genotype gypsy moth intercepts.

References

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Strontium Chloride as a Secondary Dietary Marker for Pink Bollworm Mass-Reared for Sterile Insect Technique

Lead Scientists: Michelle Walters, Nelson Foster, and Richard Zink

Team Members: John Claus, Guolei Tang, Anna Lowe, Nathan Moses-Gonzales, Dominique Ramirez, Eoin Davis, David Klein, Leighton Liesner, and Bob Staten

The APHIS-PPQ Pink Bollworm Rearing Facility rears and sterilizes pink bollworm (PBW) for release using the sterile insect technique (SIT) as a critical component of the international PBW Eradication Program. These moths are fed red dye to identify them as SIT moths. However, program personnel believe that they have trapped SIT moths with no detectable dye. These finds are not related to “hot spots” of wild moth finds or boll infestations, as would be the case if the finds were actual wild moths. The capture of a single wild-appearing moth caught in a monitoring trap may trigger eradication responses. As the program nears completion and wild moths are seldom seen, a mistaken identity is costly. Therefore, the program requested that CPHST develop a second marker for SIT moth identification.

The CPHST Phoenix Lab began work to test strontium chloride added to the PBW diet as an additional marker. PBW were reared and fed with 0, 90, 270, 540, and 720 parts per million (ppm) food-grade strontium chloride for 5 generations. No significant differences were found for diet pH, developmental time, egg production, percent hatch, pupal weight (except at 720 ppm), pupal production, percentage of successful metamorphosis from pupa to adult, adult longevity, mating, sex ratio, fecundity, deformity, or male response to pheromone in a wind tunnel or female calling (release of pheromone).

The lab worked with the Arizona State Agricultural Chemistry Lab to develop analytical methods using inductively coupled plasma-atomic emission spectrometry to measure strontium presence and quantity in a single, male, PBW moth. Adult moths were sampled at 0, 7, 14, 21, 28, 31, and 42 days posteclosion. Dietary levels of 90 and 270 ppm were not detectable in moths more than a week old. Dietary levels of 540 and 720 ppm were equally detectable. Therefore, the Rearing Facility fed all mass-reared moths 540 ppm strontium chloride in the diet for the 2012 season. Total moth production was 3.2 billion moths from April to October 2012, or about ~17 million per day. Moth production was unaffected. Lab and field tests were conducted throughout the cotton growing season. Externally dyed moths fed 0 and 540 ppm strontium were released by air and by hand over cotton fields. In all tests, the 0-ppm-strontium male PBW responded significantly better to pheromone-baited monitoring traps than 540-ppm-strontium moths.

Analysis for strontium was conducted on pupae, late instar larvae, body parts of adults, and moths aged in traps. From these analyses, we learned that:

- Strontium is readily measured in the immature forms of PBW, even when fed at 270 ppm;
- Strontium is lost between life stages;
- Strontium is primarily stored in the abdomen and excreted throughout life, as is red dye;
- Strontium is very stable in dead moths; and
- The phenomenon of dye depletion was confirmed in the field when we found moths high in strontium with no discernible red dye.

Strontium in field trapped, dye-depleted moths from the eradication program traps were also analyzed. There was sufficient strontium in 24 of 29 suspect moths trapped in Arizona to confirm that they were mass-reared. Again,

the abdomen was critical. Moths in traps may be partially eaten by predators, leaving insufficient sample material. Dissection of genitalia in the identification lab is also a problem, as it directly removes part of the abdomen and allows loss of abdominal contents. Strontium in field-trapped moths from a nonprogram area, and strontium in moths cage-reared on cotton, okra, and kenaf were also analyzed. Strontium was undetectable or found at very low levels in moths not deliberately fed strontium.

Adding food-grade strontium chloride to the PBW diet is affordable; it only added about \$8,000 to season costs for production of 3.2 billion moths. The strontium was nontoxic at 540 ppm, although there may be diminished field performance of male moths. The spectrometry method was successfully developed to analyze a single moth from a trap. The method only costs \$15 per sample and was highly reliable when tested in a timely manner (1 day) and the abdomen is present. As a result of the PBW Eradication Program's experiences in 2012, strontium will continue to be a part of the 2013 PBW Rearing Facility mass-rearing diet.

Revised Guidelines for Plant Pest Risk Assessment of Imported Fruit and Vegetable Commodities

Location: CPHST Plant Epidemiology and Risk Analysis Laboratory

Lead: Marina Zlotina

Team Members: Barney Caton, Christina Devorshak, Lisa Ferguson, Bob Griffin, Ashley Jackson, Leah Millar, Alison Neeley, and Shawn Robertson

In 2012, PERAL completed revised guidelines for plant pest risk assessment of imported fruit and vegetable commodities. These replaced the previous guidelines, which had been in use since 2000. The revised guidelines incorporate advancements in technology and pest risk assessment methods, provide a more technically correct method of assessing risk, and align PPQ's risk assessment process more closely with the International Plant Protection Convention's (IPPC) international standards for phytosanitary measures. Using the new guidelines, we will be able to produce improved pest risk assessments that are more transparent and understandable to our stakeholders.

The process of revising the guidelines began in 2009, when the revision team formed within PERAL to address issues with the 2000 version of the guidelines, improve the analytical process, and make the assessment process more efficient. The

revised guidelines consist of 10 broad processes that cover pest risk analysis (including pest listing, categorization, risk assessment, and risk management). Importantly, ratings are dynamic—increasing or decreasing as warranted—based on a close association with the “pathway of introduction,” where risk more accurately reflects each event in the pathway.

Other improvements to the guidelines include the following:

- To resolve how we addressed complex PPQ policies related to regulated pests in pest risk assessments, we now assess pests based on whether or not PPQ takes action on them at ports of entry, considering the actionable status of pests instead of their quarantine status.
- The process of analyzing the likelihood of introduction for pests is now multiplicative rather than additive. This more accurately reflects the relationship of discreet events on a pathway that would allow for the introduction of a pest. The addition of the negligible rating also allows the risk of a pest entering the United States to become negligible overall if the conditions or events required for the pest to enter the country at any particular stage of the introduction pathway are unlikely to occur.
- We now evaluate the consequences of a pest’s introduction by determining if it meets the threshold of causing unacceptable damage; pests that do not meet this threshold are not candidates for risk management.
- We now explicitly rate the uncertainty associated with each risk element.
- We developed improved guidance for determining the host status of pests.
- We revised guidance on how to provide information on risk mitigation to risk managers.

We have also added efficiencies to help reduce the amount of time it takes for analysts to produce pest risk assessments.

- We now list pests that PPQ considers nonactionable in an appendix rather than the main pest list. This reduces the amount of information analysts need to provide about those pests and means we spend much less time processing them.
- The process of analyzing a pest can stop at any one of three points: (1) if there is no endangered area in the pest risk analysis area, (2) if the pest has a negligible likelihood of introduction, or (3) if the pest doesn’t meet the threshold of being likely to cause significant consequences. Analysts are free to begin at whichever point they think may lead to stopping an analysis.

- Where scientifically justified, pests can be analyzed in ecological or taxonomic groupings, resulting in significant time savings over analyzing pests one at a time.
- Once written and reviewed, analyses completed for the consequences of introducing a specific pest in a given pest risk analysis area do not need to be updated.
- We rewrote and reformatted the document in order to present the information in a more logical, usable format.

Because we designed the revised guidelines to make the risk assessment drafting process more efficient, we expect a decrease in the time required to produce the pest risk assessments and respond to market access requests. During the creation of the revised guidelines, we gathered input from reviewers and customers in PPQ. We tested and revised the process extensively based on feedback from internal reviewers and the results of beta testing actual commodity pest risk assessments. We presented the draft guidelines to PPQ in February 2012 and sent the final version of the revised guidelines to APHIS’ Regulatory Analysis and Development staff in August 2012. A *Federal Register* notice advising the public that the revised guidelines are available and being used was published on March 6, 2013. We are currently planning to submit the revised guidelines for formal, external peer review. Also, PPQ will be highlighting and explaining the revised guidelines to industry groups that have shown an interest in past risk assessments via APHIS’ Stakeholder Registry.

Biological Control of the Winter Moth

Location: Biological Control Program and Otis Laboratory
Lead Scientist/Cooperator: Joe Elkinton, University of Massachusetts

Team Members: Vic Mastro, Ken Bloem, and Ron Weeks

Biological control is a process with multiple steps that may take 5–10 years to complete, including foreign exploration to locate potential agents, prerelease assessments of these agents to determine host range (safety) and impact, permit application for field release, development of rearing and release methodologies, implementation, and finally post-release evaluations of efficacy and possible unintended consequences. As a result, PPQ rarely conducts all of these tasks for a given project, but rather partners with other stakeholders who are also concerned with the target pest or weed. Winter moth biological control is a success story in the making that illustrates how these cooperative efforts can and should work to deliver a cost-effective program, as well

as the timeline and persistence often required to see these efforts pay off.

The winter moth (*Operophtera brumata*: Geometridae: Lepidoptera), a leaf-feeding geometrid native to Europe, invaded eastern Massachusetts around 2000 and is now causing widespread defoliation and tree death in parts of Massachusetts, Rhode Island, and Maine; it also recently invaded Connecticut. Previous invasions by this species in Nova Scotia and British Columbia, Canada, have been suppressed to very low levels by the introduction of the tachinid fly *Cyzenis albicans* from Europe. Since 2005, under permit and ongoing cooperative agreements between the University of Massachusetts and PPQ Field Operations and CPHST, collections of parasitized winter moth larvae are made and reared to the pupal stage in Canada. These pupae are then shipped to the CPHST Otis Lab quarantine facility, where they are overwintered and the flies reared out and collected for spring releases.

The first recoveries of *C. albicans* began in 2010, and flies have now been recovered from all six of the sites where releases occurred prior to 2011. Parasitized winter moth larvae have been recovered at these sites a year or more after release, proving that the flies are established. More importantly, at one of the sites in 2012, parasitism rose to approximately 30 percent. That is the point at which it should start to have a real impact on winter moth populations, based on data from Nova Scotia where parasitism rose from about 10 percent in 1959 to 70 percent 2 years later—at which point winter moth densities collapsed and have been low ever since. *Cyzenis albicans* then spread quickly over all of Nova Scotia in a very few years. We are hopeful that we are now poised to achieve similar results in New England, although it may take longer than in Nova Scotia. In the United States, there are far more extensive forests of deciduous trees, such as oak and maple, on which winter moths feed, and populations of winter moth are much larger.

Improvements to Data Management and Analysis Through the Use of Interpolated Maps To Display Capture of Sterile Mexican Fruit Flies

Location: Fruit Fly Program and Mission Lab
Lead Scientist: Pat Gomes
Cooperator: APHIS International Services

In June 2012, the CPHST Fruit Fly Program convened a technical working group in McAllen, TX, to review the Mexican fruit fly program in Texas and formulate recommendations

for improved rearing, SIT, and control and eradication efforts. The group recommended that the Mexican fruit fly program develop a biweekly report to monitor sterile fly releases and evaluate the distribution within the targeted area of release. This would help the program determine if the necessary over-flooding ratios were being maintained, as well as identify those areas where no sterile insects were being captured. Working together with APHIS International Services personnel who have geospatial information systems experience, CPHST developed maps that interpolate actual trap capture data by assigning a value related to distance. The distances are exponential values that can be adjusted and given more or less weight, resulting in more or less smoothing of the result. A graduated scale that is color-coded was developed from 0 (zero) captures in red, 1–10 captures in orange, 11–30 in yellow, 31–50 in light green, 51–150 in green, and greater than 150 in blue. This allows the program to evaluate how sterile insects are distributed over the target release blocks on a biweekly basis and make adjustments as needed. The maps were delivered for use to the program at the beginning of the fiscal year. Maps are now being used to assess the capture and distribution of the new black pupal strain of Mexican fruit fly compared with standard strains.

System for True, Accurate, and Reliable Diagnostics (STAR-D)—a Quality Management Initiative by the National Plant Diagnostic Network in Partnership With CPHST

Location: National Plant Protection Laboratory Accreditation Program
Lead Scientist: Patrick Shiel
Team Members: Kathy Burch and Geoffrey Dennis
Cooperator: Karen Snover-Clift, Cornell University

Use of quality management (QM) programs in laboratory testing is a new initiative within the plant disease diagnostic community. Several QM principles used by the human clinical, analytical, and veterinary laboratories are applicable to diagnostic testing for plant diseases. However, adaptation of several processes unique to plant diagnostics requires special review and attention. The goal of this QM initiative is to achieve functional and sustainable accreditation for diagnostic testing, while supporting continual improvement in the capabilities of plant diagnostic laboratories. CPHST is partnering with the National Plant Diagnostic Network (NPDN) to develop a quality management system called the System of True, Accurate, and Reliable Diagnostics (STAR-D).

Since the STAR-D system was initiated in 2010, QM components have been incorporated into NPDN labs, and NPDN diagnosticians have been trained as participants and as auditors. Several joint CPHST-NPDN document development and training sessions led by certified trainers occurred before 2012. In addition, documents of lab processes that incorporate QM principles were uploaded onto the NPDN Web site to serve as document templates for individual lab adoption of QM components. These document templates were adapted from those used by the American Association of Veterinary Laboratory Diagnosticians and ISO 17025-accredited labs. Success in these activities laid the groundwork for the next phase of the program, which began in 2012.

The 2012 phase of implementing and deploying a functional QM system for plant diagnostics demonstrated the integration of these activities with QM principles to allow a functional accreditation body to operate. In conjunction with the NPDN STAR-D quality management staff and CPHST personnel, several participating labs volunteered their time and effort to spearhead this phase. Several exercises were scheduled to assess the system documents with each lab's diagnostic and business procedures and their records of diagnostic processes, as well as training, equipment, and corrective actions. These exercises also served to identify potential gaps in each lab's QM system and to provide internal audit reports to further meet QM requirements. All of these exercises were preceded by additional auditor training on how to conduct an audit and document potential noncompliance issues. These auditing exercises took place as follows:

- June 19–21, 2012. Checklist Development Workshop at the University of Florida Plant Disease Clinic in Gainesville, FL.
- September 11–13, 2012. STAR-D Auditing Exercise and Gap Audit at Cornell University.
- November 6–8, 2012. STAR-D Auditing Exercise and Gap Audit at the Nevada Department of Agriculture.

Additional exercises were scheduled in 2013 as the process continued. These exercises have been showcased in several issues of the NPDN newsletter for educating the broader plant diagnostic community. A recent article written by Dr. James Stack, Director of the Great Plains Diagnostic Network, encapsulates the progress of these efforts: “The K-State Plant Diagnostic Laboratory was host to the STAR-D Audit Team for an audit exercise. I had the good fortune to participate in the opening and closing sessions where the purpose, proce-

dures, and conclusions of the audit were discussed. I left very impressed with the NPDN and USDA people tasked with the development and implementation of STAR-D, and I am confident that a functional and useful lab accreditation system will become reality... the STAR-D Accreditation Team deserves our congratulations for continued dedication to the betterment of NPDN.”

Publications

The following is a list of scientific articles published by CPHST employees in calendar year 2012.

- Barr, N.B., M.S. Islam, M. DeMeyer, and B.A. McPherson. 2012. Molecular identification of *Ceratitis capitata* (Diptera: Tephritidae) using DNA sequences of the COI barcode region. *Annals of the Entomological Society of America* 105: 339–350.
- Böröczky, K., K.E. Zylstra, N.B. McCartney, V.C. Mastro, and J.H. Tumlinson. 2012. Volatile profile differences and the associated *Sirex noctilio* activity in two host tree species in the northeastern United States. *Journal of Chemical Ecology* 38: 213–221.
- Brockerhoff, E.G., D.M. Suckling, M. Kimberley, B. Richardson, G. Coker, S. Gous, J.L. Kerr, D.M. Cowan, D.R. Lance, T. Strand, and A. Zhang. 2012. Aerial application of pheromones for mating disruption of an invasive moth as a potential eradication tool. *PLoS ONE* 7: e43767. doi:10.1371/journal.pone.0043767.
- Cooperband, M.F., K. Böröczky, A. Hartness, T.H. Jones, K.E. Zylstra, J.H. Tumlinson, and V.C. Mastro. 2012. Male-produced pheromone in the European woodwasp, *Sirex noctilio* (Hymenoptera: Siricidae). *Journal of Chemical Ecology* 38: 52–62.
- Cossé, A.A., R.J. Petroski, B.W. Zilkowski, K. Vermillion, J.P. Lelito, M.F. Cooperband, and J.R. Gould. 2012. Male-produced pheromone of *Spathius agrili*, a parasitoid introduced for the biological control of the invasive emerald ash borer, *Agrilus planipennis*. *Journal of Chemical Ecology* 38: 389–399.
- Crook, D.J., A. Khrimian, A. Cossé, I. Fraser, and V.C. Mastro. 2012. Influence of trap color and host volatiles on capture of the emerald ash borer (Coleoptera: Buprestidae). *Journal of Economic Entomology* 105: 429–437.
- Crook, D.J., K. Böröczky, K.E. Zylstra, V.C. Mastro, and J.H. Tumlinson. 2012. The chemical ecology of *Sirex noctilio*, pp. 149–158. In B. Slippers, P. deGroot and M.J. Wingfield (eds.), *The Sirex woodwasp and its fungal symbiont*. Springer Deutrecht Heidelberg, London.
- Devorshak, C. (editor). 2012. *Plant Pest Risk Analysis: Concepts and Application*. Wallingford UK: CABI. The following CPHST staff contributed chapters: R. Griffin, L. Erikson, A. Neeley, G. Fowler, Y. Takeuchi (cooperator), S. Bloem, K. Bloem, and A. Koop.
- Dodds, K.J., K.E. Zylstra, G.D. Dubois, and E.R. Hoebeke. 2012. Arboreal insects associated with herbicide-stressed *Pinus resinosa* and *Pinus sylvestris* used as *Sirex noctilio* trap trees in New York. *Environmental Entomology* 41 (6): 1350–1363.
- Domingue, M.J., J.P. Lelito, I. Fraser, V.C. Mastro, J.H. Tumlinson, and T.C. Baker. 2012. Visual and chemical cues affecting the detection rate of the emerald ash borer in sticky traps. *Journal of Applied Entomology* 137: 77–87.
- Francese, J.A., I. Fraser, M.L. Rietz, D.J. Crook, D.R. Lance, and V.C. Mastro. 2012. Relation of color, size, and canopy placement of prism traps in determining capture of emerald ash borer (Coleoptera: Buprestidae). *Canadian Entomologist* 142: 596–600.
- Koop, A., L. Fowler, L. Newton, and B. Caton. 2012. Development and validation of a weed screening tool for the United States. *Biological Invasions* 14: 273–294.
- Kuester, A.P., R.W. Jones, T.W. Sappington, K.S. Kim, N.B. Barr, R.L. Roehrdanz, P. Senechal, and J.D. Nason. 2012. Population structure and genetic diversity of the boll weevil (Coleoptera: Curculionidae) on *Gossypium* in North America. *Annals of the Entomological Society of America* 105: 902–916.

- Lyons, D.B., R. Lavalley, G. Kyei-Poku, K. vanFrankenhuyzen, S. Johny, C. Guertin, J.A. Francese, G.C. Jones, and M. Blais. 2012. Towards the development of an autocontamination trap system to manage populations of emerald ash borer (Coleoptera: Buprestidae) with the native entomopathogenic fungus, *Beauveria bassiana*. *Journal of Economic Entomology* 105 (6): 1929–1939.
- Martin, F.N., Z.G. Abad, Y. Balci, and K. Ivors. 2012. Identification and detection of *Phytophthora*: reviewing our progress, identifying our needs. *Plant Disease* 96: 1080–1103.
- Morrison, N.I., G.S. Simmons, G. Fu, S. O'Connell, A.S. Walker, D. Tarig, M. Walters, J. Claus, G. Tang, L. Jin, T. Marubbi, M.J. Epton, C.L. Haris, R.T. Staten, E. Miller, T.A. Miller, and L. Alphey. 2012. Engineered repressible lethality for controlling the pink bollworm, a lepidopteran pest of cotton. *PLoS ONE* 7: e50922.
- Paraiso, O., S.D. Hight, M.T.K. Kairo, S. Bloem, J.E. Carpenter, and S. Reitz. 2012. Laboratory biological parameters of *Trichogramma fuentesi* (Hymenoptera: Trichogrammatidae), an egg parasitoid of *Cactoblastis cactorum* (Lepidoptera: Pyralidae). *Florida Entomologist* 95: 1–7.
- Ramsey, C.L., S. Jose, and D. Zamora. 2012. Cogongrass (*Imperata cylindrica*) control with imazapyr and glyphosate combined with and without four adjuvants. *Southern Journal of Applied Forestry* 4: 204–210.
- Ryall, K.L., P.J. Silk, P. Mayo, D. Crook, A. Khimian, A.A. Cossé, K. Sweeney, and T. Scarr. 2012. Attraction of *Agrilus planipennis* (Coleoptera: Buprestidae) to a volatile pheromone: effects of release rate, host volatile, and trap placement. *Environmental Entomology* 41: 648–656.
- Ruiz-Arce, R., N.B. Barr, C.L. Owen, D.B. Thomas, and B.A. McPherson. 2012. Phylogeography of *Anastrepha obliqua* inferred with mtDNA sequencing. *Journal of Economic Entomology* 105: 2147–2160.
- Shiel, P., V. Mavrodieva, and G. Dennis. 2012. Proficiency test results summary for the 2011 National Plant Protection Laboratory Accreditation Program (NPPLAP) citrus greening (huanglongbing) detection assay. *Phytopathology* 102: S4.108.
- Suckling, D.M., E.G. Brockerhoff, L.D. Stringer, R.C. Butler, D.M. Cambell, L.K. Mosser, and M.F. Cooperband. 2012. Communication disruption of *Epiphyas postvittana* (Lepidoptera: Tortricidae) by using two formulations at four point source densities in vineyards. *Journal of Economic Entomology* 105: 1694–1701.
- Taverner, P.D., C. Sutton, N.M. Cunningham, and S.W. Myers. 2012. The control of light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) eggs on viburnum, *Viburnum tinus* L., foliage using insecticides and horticultural mineral oil sprays. *Crop Protection* 42: 83–87.
- Ugine, T.A., S. Gardescu, P.A. Lewis, A.E. Hajek. 2012. Efficacy of imidacloprid, trunk-injected into *Acer platanoides*, for control of adult Asian longhorned beetles (Coleoptera: Cerambycidae). *Journal of Economic Entomology* 105(6): 2015–2028.
- Walters, M., N.I. Morrison, J. Claus, G. Tang, C.E. Phillips, R. Young, R.T. Zink, L. Alphey. 2012. Field longevity of a fluorescent protein marker in an engineered strain of the pink bollworm, *Pectinophora gossypiella* (Saunders). *PLoS ONE* 7(6): e38547.
- Williams, D.W., K.E. Zylstra, V.C. Mastro. 2012. Ecological considerations in using *Deladenus* (= *Beddingia*) *siricidicola* for the biological control of *Sirex noctilio* in North America, pp. 135–148. In B. Slippers, P. deGroot and M.J. Wingfield (eds.), *The Sirex woodwasp and its fungal symbiont*. Springer Deutrecht Heidelberg, London.

- Yang, Z.Q., X.Y. Wang, Y.X. Yao, J.R. Gould, and L.M. Gao. 2012. A new species of *Sclerodermus* (Hymenoptera: Bethyridae) parasitizing *Agrilus planipennis* (Coleoptera: Buprestidae) from China, with a key to Chinese species in the genus. *Annals of the Entomology Society of America* 105: 619–627.
- Zylstra, K.E. and V.C. Mastro. 2012. Common mortality factors of woodwasp larvae in three northeastern United States host species. *Journal of Insect Science* 12: 83.
- The following is a list of additional publications by CPHST cooperators based on cooperative agreement work.**
- Beard, J.J., R. Ochoa, G.R. Bauman, W.C. Welbourn, C. Pooley, and A.P.G. Dowling. 2012. External mouthpart morphology in the Tenuipalpidae (Tetranychoida): *Raoiella*, a case study. *Experimental and Applied Acarology* 57: 227–255.
- Economo, E.P. and E.M. Sarnat. 2012. Revisiting the ants of Melanesia and the taxon cycle: historical and human-mediated invasions of a tropical archipelago. *The American Naturalist* 180: E1–E16.
- Fernandes, E.K.K., V.R.E.P. Bittencourt, and D.W. Roberts. 2012. Perspectives on the potential of entomopathogenic fungi in biological control of ticks. *Experimental Parasitology* 130: 300–305.
- Kane, E.C., R. Ochoa, G. Mathurin, E.F. Erbe, and J.J. Beard. 2012. *Raoiella indica* (Acari: Tenuipalpidae): an exploding mite pest in the neotropics. *Experimental and Applied Acarology* 57: 215–225.
- Kim, J.J. and D.W. Roberts. 2012. The relationship between conidial dose, moulting and insect developmental stage on the susceptibility of cotton aphid, *Aphis gossypii*, to conidia of *Lecanicillium attenuatum*, an entomopathogenic fungus. *Biocontrol Science and Technology* 22: 319–331.
- Leblanc, L., B. Fujita, S.H. Stein, and W.K. Sawamura. 2012. Trapping records of fruit fly pest species (Diptera: Tephritidae) on Oahu (Hawaiian Islands): Analysis of spatial population trends. *Proceedings of the Hawaiian Entomological Society* 44: 89–97.
- Nearns, E.H., and G. Tavakilian. 2012. New taxa and combinations in Onciderini Thomson, 1860 (Coleoptera: Cerambycidae: Lamiinae) from Central and South America, with notes on additional taxa. *Insecta Mundi* 0231: 1–24.
- Nearns, E.H., and G. Tavakilian. 2012. A new genus and five new species of Onciderini Thomson, 1860 (Coleoptera: Cerambycidae: Lamiinae) from South America, with notes on additional taxa. *Insecta Mundi* 0266: 1–23.
- Norrbom, A.L. and C.A. Korytowski. 2012. A new species of *Anastrepha* (Diptera: Tephritidae) from Colombia, Costa Rica, and Panama. *Canadian Journal of Entomology* 144: 158–168.
- Norrbom, A.L. and C.A. Korytowski. 2012. New species of *Anastrepha* (Diptera: Tephritidae), with a key for the species of the megacantha clade. *Zootaxa* 3478: 510–552.
- Phillips, B.L. and A.V. Suarez. 2012. The role of behavioural variation in the invasion of new areas. In: *Behavioural Responses to a Changing World*. Eds. U. Canolin and B. Wong. Oxford University Press. pp. 90–200.
- Rangel, D.E.N., E.K.K. Fernandes, A.J. Anderson, and D.W. Roberts. 2012. Culture of *Metarhizium robertsii* on salicylic-acid supplemented medium induces increased conidial thermotolerance, but not UV-B tolerance. *Fungal Biol-UK* 116: 438–442.
- Ristaino, J. 2012. A Lucid key to the common species of *Phytophthora*. *Plant Disease* 96: 897–903.

Sanders, N.J. and A.V. Suarez. 2012. Elton's insights into the ecology of ant invasions: lessons learned and lessons still to be learned. In: Fifty Years of Invasion Ecology: The Legacy of Charles Elton. Ed. D. Richardson. Wiley-Blackwell. pp. 239–251.

Sarnat, E.M. and E.P. Economo. 2012. The ants of Fiji. University of California Press. University of California Publications in Entomology, Volume 132. 400 pp.

Scientific Meetings

CPHST scientists participate in national and international scientific meetings, workshops, and conferences in order to promote the exchange of the latest scientific information on plant protection methods. The following is a list of such meetings attended in 2012.

- American Phytopathological Society Annual Meeting
- International Research Conference on Methyl Bromide Alternatives and Emission Reductions
- ASTM International Workshop on Dosimetry
- California Citrus Bio-Control Task Force/Citrus Research Board
- Citrus Health Research Forum
- Conference on Subtropical Biology
- Corn, Sorghum, and Soybean Seed Research Conference
- CPHST/CAPS Meeting
- Entomological Society of America Annual Meeting
- EPA Decontamination Research and Development Conference
- Food and Agriculture Organization/International Atomic Energy Association (FAO/IAEA) Research Co-ordination Workshop
- FAO/IPPC Technical Panel on Phytosanitary Treatments Annual Meeting
- IAEA Workshop on Phytosanitary Irradiation
- Imported Fire Ant and Invasive Pest Ant Research Conference
- International Congress of Entomology (ICE) Agricultural Acarology Symposium
- International Cotton Pest Work Committee
- International Symposium on Invasive Plants and Global Change
- International Workshop for *Phytophthora*, *Pythium*, and *Phytophythium*
- IPPC Technical Panel on Diagnostic Protocols
- Korean Society of Plant Pathology International Conference
- Midwest Weather Working Group
- National Cotton Council Technical Action Committee
- National Grasshopper Management Board Meeting
- National Gypsy Moth Program Review
- North American Chemical Residue Workshop
- North Central Forest Pest Workshop
- North Dakota Weed Control Association
- Opportunities in Phytosanitary Irradiation Workshop
- Society for Invertebrate Pathology Annual Meeting
- Society of Risk Analysis Annual Meeting
- Southern Section of the Association of Analytical Communities International
- Texas Citrus Showcase
- Texas and Florida Scientific and Grower Exchange
- Universal Plant Virus Microarray Workshop
- USDA Hispanic Serving Institutions Project Directors Conference
- Weed Science Society of America Annual Meeting
- Wyoming Weed and Pest Council

Appendix A. Funded Projects for Fiscal Year 2012

Funding Category	\$ in thousands
AQI-User Fee	13,655
Farm Bill	11,716
Plant Methods	7,330
Biocontrol	3,339
Emerald Ash Borer	1,290
Pest Detection	1,192
Fruit Fly	697
Asian Longhorned Beetle	620
Sirex	582
Grasshopper	573
European Grapevine Moth	390
Sudden Oak Death	249
Imported Fire Ant	205
Light Brown Apple Moth	201
Gypsy Moth	160
Citrus Health Response Program	150
Select Agent	150
Cotton Pests	100
Golden Nematode	20
Total	42,619

Funding lines for CPHST in fiscal year 2012. Includes allocated funds of \$30.9 million and Farm Bill Section 10201 funds of about \$11.7 million.

AQI Laboratory and Biloxi Station

- Lure shelf life and quality assurance determination (USDA-ARS)
- Outsourcing residue analysis on APHIS eradication and suppression program samples (USDA-AMS)
- Improving techniques for detection of prohibited plants and invasive pests at ports of entry (University of Florida)
- Commodity Treatment Information System maintenance (NCSU)
- Evaluating biomarkers for verifying successful phytosanitary irradiation treatments of insect pests in commodities in both normal and modified atmospheres (University of Florida)
- Commodity source identification using inductively coupled plasma mass spectrometry (DHS)

- Development of grass sod treatments for imported fire ant quarantine (Auburn University, University of Arkansas)
- Development of imported fire ant quarantine treatments for in-field and balled-and-burlapped nursery stock (USDA-ARS, Tennessee State University)
- Biological control of the imported fire ant using phorid flies: cooperative rearing and release program (Florida Division of Plant Industry, USDA-ARS)
- Development of rapid imported fire ant assay kit and species specific surveillance trap (USDA-ARS)
- Identification and survey collaboration with the Coastal Research and Extension Center, Biloxi, MS (MSU)

Beltsville Laboratory

Plant Pathogen Methods Development

- A novel virus of the genus *Cilevirus* causing symptoms similar to citrus leprosis
- Evaluation of DAS-ELISA for the detection of CiLV-C
- Molecular diagnostics methods for the sweet orange scab fungal pathogen: *Elsinoë australis*
- Molecular diagnostics methods for the late wilt of corn fungal pathogen: *Harpophora maydis*
- Molecular diagnostics methods for the *Phytophthora kernoviae*: Markers for differentiation of isolates from Great Britain and New Zealand
- Multiplex real-time PCR methods for detection and identification of nematodes *Anguina funesta*, *A. agrostis*, *A. tritici*, and *A. pacificae*
- Development of real-time PCR Methods for detection and identification of *Rathayibacter toxicus*
- Two additional genes for advanced detection of *Candidatus Liberibacter asiaticus* associated with citrus HLB

Biotechnology Platforms Evaluation/Adaptation

- Evaluation and adaptation of CANARY technology for rapid detection of *Phytophthora*
- Evaluation and adaptation of the LiNK technology for rapid extraction of plant pathogen DNA

Proficiency Testing for the NPPLAP

- Test panel development for HMB, *Plum pox virus*, and *P. ramorum*

Diagnosis of Regulated Plant Pathogens

- Complete diagnoses of *Plum pox virus* suspect samples and other plant pathogens

Quality Management/Quality Assurance

- Release work instructions to be used in PPQ diagnostic processes

Fort Collins Laboratory

Biological Control

- Survey for natural enemies of Canada thistle (*Cirsium arvense*) (CABI Bioscience United Kingdom)
- Biological control of Canada thistle
- Biological control of Russian knapweed
- Biological control of field bindweed
- Biological control of yellow toadflax and garlic mustard
- Biological control of hoary cress
- Biological control of hound's-tongue
- Biological control of dyer's woad and perennial pepperweed
- Biological control of hawkweeds
- Risk assessment and monitoring of target and non-target plant utilization by the hound's-tongue root weevil *Mogulones crucifer* in northern Washington and Idaho (University of Idaho)
- Development of a database: biocontrol agents of insect pests released in the United States (Colorado State University)

Decontamination and Waste Disposal

- Assessment of disinfectants for control of *Phytophthora ramorum* (Colorado State University)
- Sterilization and disposal of agricultural quarantine waste (Kansas State University)
- Rapid, automated molecular identification system for prohibited plants (University of Alabama)
- Soil sanitation for Benghal dayflower and soybean cyst nematode control (University of Florida)

Digital Identification Tools and Resources

- *Anastrepha* and *Toxotrypana*: Descriptions, Illustrations, and Interactive Keys (USDA-ARS, Universidad de Panamá, CSIRO Australia), <http://delta-intkey.com/anatox/intro.htm>
- Antkey (University of Illinois), www.antkey.org

- AphID: Identification Guide for Cosmopolitan and Polyphagous Aphid Species (University of Maryland, University of Montreal, USDA-ARS), <http://aphid.aphidnet.org>
- Citrus Pests (University of Florida, Southern Plant Diagnostic Network), <http://idtools.org/id/citrus/resource>
- Flat Mites of the World (University of Maryland, USDA-ARS, Queensland Museum), <http://idtools.org/id/mites/flatmites>
- Hispines of the World (Smithsonian), <http://idtools.org/id/beetles/hispines>
- IDpic (Colorado State University, University of Georgia)
- IDpic Node (Colorado State University, University of Georgia), www.ipmimages.org/browse/NodeThumb.cfm?Node=5
- Longicorn ID: Tool for Diagnosing Cerambycoid Families, Subfamilies, and Tribes (University of New Mexico, USDA-ARS, CSIRO Australia), <http://cerambycids.com/longicornid>
- Table Grape Spider ID (UC-Riverside), http://idtools.org/id/table_grape/spider/login.php
- TortAI: Tortricids of Agricultural Importance (Colorado State University, CDFA), <http://idtools.org/id/leps/tortai>

Spatial Technologies

- Evaluate and implement a Web-based application for sharing geospatial data using historic barberry eradication data (Farm Bill, Washington State University)
- AGM predictive model to assist surveillance and early identification
- Observation sampling analysis to support an area-wide monitoring study for ACP in the Lower Rio Grande Valley, TX

Survey Support

- CPHST science support for CAPS surveys of pathogens, nematodes, and weeds
- Confirming the pathogenicity and host range of *P. ramorum* (Farm Bill-funded, Colorado State University, UC-Berkeley)
- Rapid and accurate diagnostic identification of phytoplasmas (Farm Bill-funded, University of Florida, USDA-ARS)
- Rapid and accurate detection and identification of palm viroids (Farm Bill-funded, University of Florida, USDA-ARS)

Weed Management

- Efficacy screening for synthetic and organic herbicides on giant hogweed resprouts
- Common tansy herbicide field study
- Reducing Benghal dayflower seedbank populations
- Database development for localized spread rates for three invasive weeds

Mission Laboratory

Fruit Fly Program Support

- Developing Mexfly diet formulations
- Mexfly new strain development
- Implementation of the incubation of Mexfly eggs by bubbling in the Mexican Fruit Fly Rearing Facility
- Assessment, identification and suppression of fungal contaminants in the Mexican Fruit Fly Rearing Facility

Integrated Pest Management

- Operation of the CPHST Mission Arthropod Quarantine Facility
- Biological control of *Harrisia cactus* mealybug in Puerto Rico

Molecular Diagnostics

- Molecular identification tools for the Asian gypsy moth (University of Texas-Pan American)
- Molecular diagnostics of native nematodes and their potential as biocontrol agents against *Sirex noctilio* (Southern Illinois University)
- Population genetics of exotic pest fruit flies (University of Texas-Pan American)
- Development of molecular diagnostic techniques for mollusks of economic importance to American agriculture
- Development of molecular techniques that identify cryptic thrips species
- Molecular pathway analysis of *Anastrepha* fruit flies
- Fruit fly molecular identification program to support diagnostics and pathway analysis
- Development of diagnostic tools for identifying species and geographic sources of intercepted Dacinae fruit flies
- Development of molecular diagnostic techniques that identify foreign fruit fly pests
- Diagnostic resources to support fruit fly exclusion and eradication (Farm Bill-funded, USDA-ARS)

- Molecular pathway analysis of citrus greening disease (Farm Bill-funded, University of Texas-Pan American)
- Enhancement of fruit fly larval identification and taxonomy (Farm Bill-funded, Florida Department of Agriculture and Consumer Services)
- Development of molecular DNA barcoding tools to identify fire ants (Farm Bill-funded, University of Illinois)

Citrus Health

- Areawide management of Asian citrus psyllid (Farm Bill-funded)
- Population genetic analysis of *Diaphorina citri* and *Tamarixia radiata* (Farm Bill-funded)
- Survey for *Brevipalpus* mites and Citrus Leprosis Virus in Texas and Limes Imported from Mexico (Farm Bill-funded)
- Evaluating the biological control of Asian Citrus Psyllid in the Rio Grande Valley of Texas (Farm Bill-funded)
- Studies on sweet orange scab caused by *Elsinoe australis* (Farm Bill-funded)

Otis Laboratory

Pest Survey and Detection

- Odor-based detection and monitoring systems for exotic pests
- Development of survey tools
- Survey and regulatory technology for European grape berry moth (*Eupocelia ambigua*)
- Improved analysis and interpretation of insect trapping data
- Molecular diagnostics of exotic pests
- Trap and lure for walnut twig beetle (Farm Bill-funded)
- Development of Lepidoptera attractants (Farm Bill-funded)
- *Tuta absoluta* pheromone detection and management tools (Farm Bill-funded)
- Attraction and detection of the polyphagous shot hole borer, *Eumallacea* sp. (Farm Bill-funded)
- Attraction and detection of the fruit piercing moth (Farm Bill-funded)
- Development of attractants and improved trap designs for exotic wood borers (Farm Bill-funded)
- Smart trap/remote sensor development (Farm Bill-funded)

Phytosanitary Treatment Development

- Alternative quarantine treatments for invasive hitchhiking snails

- Fumigation as a control for wood boring insects in solid wood packing
- Japanese beetle regulatory treatments
- Development of regulatory treatments for ALB
- Methyl bromide alternative wood and log treatments for export (EAB and pathogens)
- Validation of treatment options
- Methyl bromide alternative treatments for unprocessed wood and log exports (Farm Bill-funded)
- Alternative regulatory treatments for LBAM
- Phosphine gas in combination with cold treatment and gamma radiation as a methyl bromide alternative for imported fruits
- Survey, detection, and treatment evaluation on insects associated with wood packaging materials
- Development of regulatory tools for thousand cankers disease (Farm Bill-funded)
- Development of postharvest treatments quarantine treatments for *Lobesia botrana* and other pests (Farm Bill-funded)
- Regulatory treatments for exotic high risk fruit flies that regularly or are likely to invade the United States (Farm Bill-funded)
- Development of new treatment options for khapra beetle (Farm Bill-funded)

Forest Pest Management

- Behavior, biology, and control of wood borers (University of Massachusetts)
- Evaluation of soil applications of systemic insecticides for control of wood-boring beetles
- Evaluation of pesticide residues, levels, and distribution in trees treated with systemic insecticides
- Forecasting pest potential through offshore assessments
- Production of insect diets and life stages for use in APHIS and other cooperative research programs
- Evaluation of systemic insecticides in China
- Evaluation of insect preference and suitability of tree species as insect hosts
- Predicting the cumulative and ecological impacts of exotic pests and pathogens on the eastern United States (Farm Bill-funded)
- Characterizing risk posed by exotic Lymantrids (Farm Bill-funded)

Emerald Ash Borer

- EAB alternative control strategies—biological control
- Distribution of EAB in China

- Ecology of EAB
- Support for the EAB program

Asian Longhorned Beetle

- Improved rearing technology for ALB
- Insecticide control of exotic wood borers (EAB and ALB)
- Infestation dynamics of ALB and EAB in North America
- Support for the ALB program

Gypsy Moth

- Molecular analysis of male gypsy moths trapped in U.S. ports and other high-risk sites
- Support for the Russian/Japanese/Chinese/Korean exotic *Lymantria* exclusion program
- Production of nuclear polyhedrosis virus for gypsy moth control

Sirex woodwasp

- Behavior and sensory ecology of *Sirex noctilio* for improvement of detection and surveillance
- Development of monitoring tools for *Sirex*
- Development of degree day model for *Sirex*
- Biological control of *Sirex* using entomopathic nematodes

Light Brown Apple Moth

- Improved trapping methods for LBAM
- Optimizing mating disruption for LBAM
- Production of lures for LBAM national survey
- Assess insecticides for LBAM management
- Population dynamics and ecology of LBAM

European Grapevine Moth

- Support for the EGVM program
- Radiation biology of EGVM (Farm Bill-funded)

California Station

- Monitoring and control of *Lobesia botrana*, EGVM (Farm Bill-funded, UC)
- Research on postharvest and regulatory control treatments for EGVM (Farm Bill-funded, UC)
- Laboratory screening and field testing to identify and develop better compounds for trapping of EGVM (New Zealand Plant and Food)
- Development of control technologies and strategies for management of phytosanitary risks for invasive species affecting agriculture in the United States: LBAM, EGVM, spotted wing drosophila, and Asian citrus psyllid (NCSU)

- Development of an integrated pest management program for LBAM in nurseries (Farm Bill-funded, UC)
- Irradiation as a phytosanitary treatment of Lepidoptera pupae (USDA-ARS, Foreign Agricultural Service)

Phoenix Laboratory

Rangeland Grasshopper/Mormon Cricket

- Development of a new active ingredient in bait and evaluation of a new formulation of the traditional active ingredient in bait for controlling grasshopper and Mormon cricket on rangeland
- Confirming an effective dose of chlorantraniliprole (Prevathon) for control of rangeland grasshoppers
- Continued operational scale evaluation of EDT Concentrate as a replacement for oil diluents in Dimilin spray mixes to control rangeland grasshoppers
- Evaluation of ground applications of selected domestic strains of pathogenic fungi, on four hectare (10 acre) and mini plots, for control of rangeland grasshoppers (Orthoptera: Acrididae) near Dagmar, MT
- Collection, cataloging, and screening of domestic fungi pathogenic from soil samples (Utah State University)
- Continue to acquire, catalog, and make available field research data, historical documents, and limited distribution literature that documents the history of grasshopper and Mormon cricket control efforts conducted by PPQ

Pink Bollworm

All work is conducted with support from cooperators at the Arizona Cotton Research and Protection Council.

- Evaluation of strontium chloride as an additional dietary marker for PBW in a sterile release program
- Measurement of the impact of irradiation dose on PBW field performance and potential for progeny
- Preparation and shipment of insect diet to cooperators
- Evaluation of effect of storage on field efficacy of PBW lure
- Development of method for high performance liquid chromatography to detect dietary markers in field-trapped insects to separate mass-reared from wild moths
- Development and maintenance of bibliographies to gather and house documents regarding current research topics
- Rearing, documentation, and shipping of genetically modified PBW used as quality assurance specimens in the PBW Eradication Program

Plant Epidemiology and Risk Analysis Laboratory

Commodity Pest Risk Analysis

- Produce scientific documentation in support of trade decisions regarding the importation of commodities
- Prepare pest risk assessments, identify and evaluate potential mitigations, and review pest risk assessments prepared by other countries
- Respond to scientific and technical issues associated with commodity import rulemaking
- Identify and develop improvements in the pest risk assessment and risk management process

Exports

- Provide scientific and analytical support to facilitate new market access for U.S. agriculture exports
- Provide scientific and analytical support to the expansion or maintenance of export opportunities that are blocked by technical barriers
- Prepare Export Risk Analysis products (focus is on pest lists of arthropods and plant pathogens) associated with commodities for export
- Provide scientific information and analytical support for trade dispute settlement

Risk Analysis for Individual Organisms and Pathways

- Through the New Pest Advisory Group (NPAG), assess new and imminent pest introductions into the United States and make recommendations to PPQ management regarding appropriate PPQ responses to exotic plant pests, including arthropods, mollusks, pathogens, and weeds
- Perform Deregulation Evaluations for Established Pests (DEEP) to support policy decisions on pest status for consistency with import actions
- Produce scientific documentation in support of trade decisions, inspection activities, or monitoring in relation to specific identified pathways of interest by which exotic plant pests may become established in the United States

Accreditation and Certification of Risk Analysis Functions

- Through audits and improvements, maintain ISO certification for the Lab's commodity risk assessments and NPAG

Outreach and Training/Capacity Building/Regulatory Curricula

- Provide instructors for a regulatory science minor at NCSU
- Maintain the strong cooperative relationship established between CPHST, NCSU, and other academic institutions
- Support a regulatory curriculum that provides training to students in relevant fields on key aspects of regulatory plant protection
- Host risk analysts from other countries, pairing visiting scientists with resident analysts as mentors to provide training in risk analysis methods

Plants for Planting (Q-37) Analyses and Regulatory Overhaul

- Provide scientific and strategic support to revise and update 7 CFR 319.37, the quarantine that regulates the import of plants for planting
- Advance the regulatory process through the development of methodologies and analyses to support the APHIS decision-making processes associated with the evaluation of pest risk prior to authorizing the entry of propagative material into the United States
- Prepare risk assessments for propagative material proposed for importation

International Standards: IPPC, NAPPO

- Provide leadership and expertise to the Harmonization Advisory Group, a new cross-functional team, on matters related to international standard setting
- Lend support, time, and expertise to international organizations, such as the IPPC and NAPPO, by participating on international working groups to write standards and review draft standards and specifications for new standards as they become available
- Manage and maintain the Web site for the Phytosanitary Alert System (PAS) Panel, which provides oversight to early warning initiatives for NAPPO

Information Systems and Biosurveillance Analysis Forecasting

- Maintain and expand the Global Pest and Disease Database (GPDD)
- Through the NCSU/APHIS Plant Pest Forecast (NAPPEFAST) System, support the predictive pest-mapping needs of the CAPS program and the risk assessment activities of the Lab

- Generate Global Plant Hardiness Maps and post them on the NAPPEFAST Web site, www.nappfast.org
- Create risk maps for the CAPS Top 50 Pests, as well as for CAPS 2010, CAPS historical pests, and CAPS commodity surveys
- Support the CAPS Program by producing pest prioritization lists using the analytic hierarchy process
- Produce and circulate the Exotic Pest Information Collection and Analysis (EPICA) notifications

Weed Risk Assessment

- Generate pest lists for weeds
- Conduct weed risk analysis
- In support of the Q-37 revision, revise weed risk assessment guidelines to improve and streamline the process
- Conduct weed risk assessments of plants that pose a risk to the United States as defined by the Plant Protection Act of 2000
- Develop training for staff and others using new weed risk assessment guidelines
- Develop and maintain status as experts on weed risk assessment
- Provide training, information, consultation, and analyses to Federal, State, and public customers, especially in relation to noxious weed issues

Reference Management

- Maintain and improve physical library (currently 4,000 scientific references, including publications, large-scale maps, and videos)
- Maintain and improve PRA library in Endnote (currently 600 PRAs in PDF format that are indexed and text-searchable)
- Maintain and improve the digital library of scientific references (currently 14,000 documents in PDF format housed in an Endnote library)
- Maintain and improve the Equal Employment Opportunity library (83 topical books and videos are available to CPHST employees through a SharePoint request site)
- Maintain and improve the health and fitness library (currently two shelves of books and videos)

Biological Control Program

A full listing of the project titles and publications of CPHST scientists working on biological control can be found under the highlights and funded projects sections for the individual

CPHST laboratories, with the exceptions of Amy Roda and Scott Weihman at the Miami Station and Pedro Rendón at the Guatemala Station, who are administered from the Director's Office in Raleigh, NC.

Coordinator Cooperative Agreements

- Developing biological control and other safeguarding tools to manage invasive pests (Florida A&M University)
- Understanding the economics of biological control (Florida A&M University)
- Laplap (kudzu) bug biological control (University of Georgia)

Farm Bill-Funded Projects

- Biological control of the brown marmorated stinkbug (USDA-ARS)
- Biological control of the brown marmorated stinkbug (University of Delaware)
- Biological control of the brown marmorated stinkbug (Michigan State University)
- Biological control of the brown marmorated stinkbug (Oregon Department of Agriculture)
- Biological control of the brown marmorated stinkbug (FDACS)
- Biological control of the brown marmorated stinkbug (MSU)
- Cactus moth mating disruption (USDA-ARS)
- Disruption of cactus moth larval trail following systems (State University of New York)
- Cactus moth biological control (USDA-ARS)
- Cactus moth biological control (FDACS)
- Cactus moth biological control (MSU)
- Enhanced mitigation techniques for control of several whitefly species (FDACS)
- Enhanced mitigation techniques for control of several whitefly species (University of Florida)
- ACP biological control in California (California Citrus Research Board)

Miami Station

- Identifying native natural enemies of the red bay ambrosia beetle
- Assessing biological control options for Harrisia cactus mealybug in Florida and Puerto Rico
- Designing methods to trap giant African snails in urban and suburban settings
- Offshore safeguarding in the Caribbean: developing improved trapping protocols for *Tuta absoluta* and

understanding host plant relationships of *Anastrepha grandis* in Panama

- Will natural enemies and winter temperatures prevent *Mikania micrantha* from becoming invasive? (University of Florida)
- Land snail aggregation pheromones: A tool to detect and control giant African snail (UC-Riverside)
- Mitigating invasive pests in Puerto Rico: A front line initiative for rapid response (Farm Bill-funded)

Guatemala Station

- Developing mass-rearing techniques for the Medfly egg parasitoid *Fopius ceratitivorius*
- Field testing augmentative releases of *Fopius ceratitivorius* in combination with SIT for Medfly control

Fruit Fly Program

Headquarters

- CPHST Quality Assurance Survey Program in support of national fruit fly trap and lure procurement
- Fruit systems approaches and regulatory treatments using foliar sprays
- Fruit fly host compendium
- Alternatives to diazinon ground drench for fruit fly control

Florida Fruit Fly Methods Support

- Field tested CeraTrap liquid attractant for sterile Medflies and Caribbean fruit fly
- Estimated fruit fly detection probabilities using software
- Improved efficacy of traps and lures
- Evaluated single swath aerial releases for Mediterranean fruit fly
- Evaluated post-knockdown quality control procedures
- Analyzed sterile release population suppression with the Caribbean Fruit Fly Free Certification Program managers
- Evaluated and monitored production quality
- Coordinated bait station trials
- Evaluation of protein diet augmentation
- Evaluation of agar feeding block hydration
- Evaluation of sterile fly eclosion tower holding times
- Optimal sterile fly adult chill times
- Evaluation of combined three-component Bio-Lure cones versus patches
- Evaluation of solid male fruit fly lures versus liquids in Florida
- Fruit fly invasion modeling and eradication program support

Mexican Fruit Fly Program

- Developed egg-bubbling and handling procedures
- Determined best artificial lure to use in Mexfly monitoring and trapping
- Mexican Fruit Fly Sterile Insect Monitoring Model
- Methods for detecting and combatting two *Morganella* bacterial isolates
- Effect of loading individual trays in Worley tower system at different pupal densities

Hawaii and California Fruit Fly

- Hawaiian Pacific Basin Center for Insect Control
- Mark-release-recapture studies of *Bactrocera cucurbitae* and *B. dorsalis* in Orange County, CA
- Trapping studies of wild *B. cucurbitae* and *B. dorsalis* with liquid or solid formulations of male lures
- Trapping study comparing the effectiveness of melolure to cue-lure in capturing *B. cucurbitae*
- Distance-dependent capture probabilities of *B. cucurbitae* and *B. dorsalis*
- Effects of chill duration on flight ability in sterile male Medflies
- Mark-release-capture of sterile male Medflies to describe postrelease dispersal
- Effects of prerelease exposure to methyl eugenol on the mating competitiveness of sterile males of *B. dorsalis*
- Distribution patterns of flies released using current aerial release protocols
- Effect of chilling and the knockdown procedure on sterile Medflies
- Morphological training on identification of economically important *Bactrocera* and *Dacus* species

Guatemalan Moscamed Operations Support for Fruit Fly Preventative Release and Eradication Programs

- Field evaluations for quality assurance of traps and lure products used in fruit fly programs
- Alternatives to replace the use of agar to feed sterile Medflies
- Fabrication and testing of bait stations for use in fruit fly programs to reduce the need for ground bait sprays
- Evaluation of bait station efficacy and shelf-life
- Evaluation of diet ingredients for Medfly production at El Pino Medfly Rearing Facility in Guatemala
- Development and use of a monitoring tool to calculate release densities based on sterile to fertile fly ratios
- Field cage evaluations of different strains of Mexfly

Office of the Executive Director

Farm Bill-Funded Cooperative Agreements

- Engineering mobile RNA in Carrizo rootstock to create a hypersensitive programmed cell death response in mature scions to control citrus greening (Integrated Plant Genetics, Inc.)
- Disease modeling via stochastic simulation to test disease control and mitigation strategies to maximize regulatory intervention (University of Cambridge)
- Development of reagents and diagnostic tools for the detection of plant pathogens of citrus (University of Florida)
- Transmission of *Xylella fastidiosa* to sweet orange seedlings through infected seed (USDA-ARS)
- Stochastic modeling and the design of early detection surveys for high-risk pathogens (Rothamsted Research, United Kingdom)
- The effect of physical and chemical treatment on activity of citrus canker lesions and other pests and diseases on fruit and leaves of citrus (University of Florida)
- Methods development for routine assessment of HLB susceptibility (University of Florida)
- Determination if *Ficus carica*, *Psidium guajava*, *Pisonia aculeata*, *Trichostigma octandrum*, and *Cleome rutidosperma* are alternative hosts of *Candidatus Liberibacter asiaticus*, the associated bacterium of Huanglongbing (University of Florida)
- Development of field insectary cage rearing methods for parasitoids of ACP (Citrus Research Board of California)
- Predictive mapping to inform citrus producers of HLB/ACP risk to improve targeting of control efforts based on repeated statewide survey (Citrus Research Board of California)
- Detection, identification and genetic analysis of *Candidatus Liberibacter asiaticus* (USDA-ARS)
- Prevention of the spread of citrus black spot outside of southern Florida (University of Florida)
- Understanding *Guignardia citricarpa* ascospore production and potential inoculum reduction strategies in Florida (University of Florida)
- The National Ornamentals Research Site at Dominican University of California (Dominican University of California, Oregon State University, University of California, Washington State University, USDA-ARS)
- Validating commercial formulation of *Trichoderma asperellum* against *P. ramorum* infested soil (USDA-ARS)

- Testing of fungicides and disinfectants on Gladiolus rust along with host resistance (Rutgers University, USDA-ARS)
- Study of *Uromyces transversalis* mycelia and development of uridinia, development and testing of antibody assays (Rutgers University, USDA-ARS)
- Canine detection of *Phytophthora ramorum* (USDA-ARS)
- Alternative methods of eradication for potato cyst nematode (University of Idaho, Oregon State University, USDA-ARS)
- Chrysanthemum white rust efficacy, overwintering, latency, host susceptibility, and isolate variability (Rutgers University, University of Georgia, USDA-ARS)

Appendix B. Acronyms and Abbreviations

ACP	Asian citrus psyllid
AGM	Asian gypsy moth
AHP	analytical hierarchy process
ALB	Asian longhorned beetle
AMS	Agricultural Marketing Service
AQI	agricultural quarantine and inspection
APHIS	Animal and Plant Health Inspection Service
ARS	Agricultural Research Service
CANARY	Cellular Analysis and Notification of Antigen Risk and Yield
CAPS	Cooperative Agricultural Pest Survey
CBP	Customs and Border Protection
CCST	CPHST CAPS Support Team
CDFA	California Department of Food and Agriculture
CiLV	Citrus leprosis virus
CiLV-C	Citrus leprosis virus-cytoplasmic
CPHST	Center for Plant Health Science and Technology
DEEP	Deregulation Evaluation for Established Pests
DHS	U.S. Department of Homeland Security
EAB	emerald ash borer
EGVM	European grapevine moth
EPA	U.S. Environmental Protection Agency
EPICA	Exotic Pest Information Collection and Analysis
FDACS	Florida Department of Agriculture and Consumer Services
FAO	Food and Agriculture Organization
FY	fiscal year
GPDD	Global Pest and Disease Database
GSA	General Services Administration
HCM	Harrisia cactus mealybug
HLB	<i>huanglongbing</i> (citrus greening)
IAEA	International Atomic Energy Association
IFA	imported fire ant
IPPC	International Plant Protection Convention
ISO	International Organization for Standardization
ISPM	International Standard for Phytosanitary Measures
ITP	Identification Technology Program
LBAM	light brown apple moth
LiNK	Lincoln Lab Nucleic-Acid Kit
Medfly	Mediterranean fruit fly
Mexfly	Mexican fruit fly
MSU	Mississippi State University
NAPPEFAST	NCSU/APHIS Plant Pest Forecasting System
NAPPO	North American Plant Protection Organization
NCSU	North Carolina State University
NIS	National Identification Services
NORS-DUC	National Ornamental Research Site at Dominican University of California
NPAG	New Pest Advisory Group
NPDN	National Plant Diagnostic Network

NPPLAP	National Plant Protection Laboratory Accreditation Program
NSTP	National Scientific Technologies Program
PBW	pink bollworm
PCR	polymerase chain reaction
PERAL	Plant Epidemiology and Risk Analysis Laboratory
PIS	Plant Inspection Station
PPQ	Plant Protection and Quarantine
PRA	pest risk analysis
QM	quality management
RT-PCR	reverse transcription PCR
SIT	Sterile Insect Technique
S&T	Science and Technology
STAR-D	System for True, Accurate, and Reliable Diagnostics
UC	University of California
USDA	United States Department of Agriculture

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