USDA

United States Department of Agriculture

Animal and Plant Health Inspection Service

Safeguarding Through Science

Center for Plant Health Science and Technology

2010 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: *Gratiana boliviana* biocontrol organism feeding on the noxious weed tropical soda apple *(USDA/Agricultural Research Service, Peggy Greb)*

Message from the Director

The Center for Plant Health Science and Technology (CPHST) provides scientific support for the regulatory decisions 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 is responsible for ensuring that PPQ has the information, tools, and technology to make the most scientifically valid regulatory and policy decisions possible. In addition, CPHST ensures that PPQ's operations have the most scientifically viable and practical tools for pest exclusion, detection, and management. I lead and monitor this effort with the support and expertise of Bill Dickerson, the Associate Director for CPHST. This 2010 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 long-term strategic goals.

The organization consists of approximately 250 employees in 8 labs, 4 units, and multiple work stations in the United States, Colombia, and Guatemala. The new CPHST laboratory in Miami, FL, which is expected to officially open in the fall of 2011, will provide leadership in commodity treatment and port technology.

Our work is organized into six National Science Programs: Agricultural Quarantine Inspection and Port Technology; Risk and Pathway Analysis; Domestic Surveillance, Detection, and Identification; Response and Recovery Systems Technology (RRST)-Arthropods; RRST-Plant Pathogens and Weeds; and RRST-Citrus Health. Our scientists provide leadership and expertise in a wide range of fields, including risk assessments that support trade, commodity quarantine treatments, pest survey and detection methods, molecular diagnostics, and integrated pest management.

Some highlights of significant CPHST efforts in 2010 include:

Citrus Health. CPHST has created a Citrus Health National Science Program in response to the recent surge in citrus pest invasions that are threatening the citrus industry. The complex science coordination needed to address citrus canker, citrus greening, citrus black spot, sweet orange scab, and overall citrus health requires a dedicated program for this effort. Recent CPHST products have had important impacts on the citrus industry. For example, CPHST:

- Conducted an areawide management pilot project that demonstrated that Asian citrus psyllids, which transmit citrus greening, can be reduced by more than 90 percent through appropriate insecticide applications and other management methods. The success of the pilot project provided the framework to begin a grower-initiated areawide management program in Texas.
- Quickly validated emergency-use diagnostic protocols for sweet orange scab and citrus black spot when these pathogens were detected in the United States, providing critical methods for survey and regulatory activities.
- Conducted risk assessments for citrus black spot and sweet orange scab, concluding that fresh fruit is not a pathway for disease spread if the fruit undergoes certain packinghouse procedures. This information was used to update regulatory requirements and provided conditions to allow interstate movement of citrus outside quarantine areas.
- Provided critical risk assessments and technical support for the recently released interim rule for citrus nursery stock.

Fruit Fly Unit. In order to manage the numerous projects related to fruit fly detection and eradication, CPHST has established a Fruit Fly Unit that is headed by a central coordinator. In addition, two staff members were hired specifically to support local survey and eradication efforts in Florida and California. Examples of recent significant projects for this unit include:

- Developing a Sterile Insect Technique (SIT) and Eclosion Quality Assurance Web site to compile and analyze fruit fly rearing and release data for facilities in Florida, California, Texas, and Guatemala.
- Initiating a project to conduct molecular diagnostics on fruit fly larvae intercepted at ports of entry to assist in determining the species' identity and geographic origin.
- Developing a novel bait station that is effective and inexpensive and can be used in environmentally sensitive areas.

European Grapevine Moth (EGVM). In the fall of 2009, EGVM was detected in California, and survey activity has shown that the moth is present in several grape-producing counties. This pest has the potential to severely impact the wine and grape industry. There was a critical need to quickly develop management methods to protect production and commodity treatment methods to protect trade. CPHST's response included:

- Leading a technical working group to provide information on issues regarding trapping lures, mating disruption, treatments, and hosts.
- Producing over 250,000 lures for EGVM survey activities.
- Developing post-harvest treatments for EGVM to allow the safe movement of California nursery stock and fruit. These treatments have also allowed uninterrupted shipment of grapes from Chile into the United States.
- Initiating work to develop areawide control methods for EGVM, including evaluating the use of mating disruption and timed pesticide treatments.

Farm Bill Section 10201 Projects. CPHST has been able to leverage significant additional support through Farm Bill Section 10201, which provides funds for plant pest detection and surveillance, threat identification and mitigation of plant pests and diseases, and the development of certification systems and nursery plant pest risk management systems. In fiscal year (FY) 2010, CPHST directed 74 Farm Bill projects, with funding totaling over $8.3 million. This funding allowed CPHST to initiate internal and cooperative projects in areas such as pest survey technology, identification tools, molecular diagnostics, citrus pest management methods, and nursery pest management methods. CPHST has received Farm Bill funding of over $10 million in FY 2011 to continue this work and initiate new projects.

CPHST is recognized nationally and internationally for its leadership in scientific development to battle plant pests and diseases. We are proud to serve in this role and are issuing this annual report to provide an informative overview of CPHST's many accomplishments and projects.

Dr. Philip Berger, Director

Center for Plant Health Science and Technology

USDA-APHIS-PPQ

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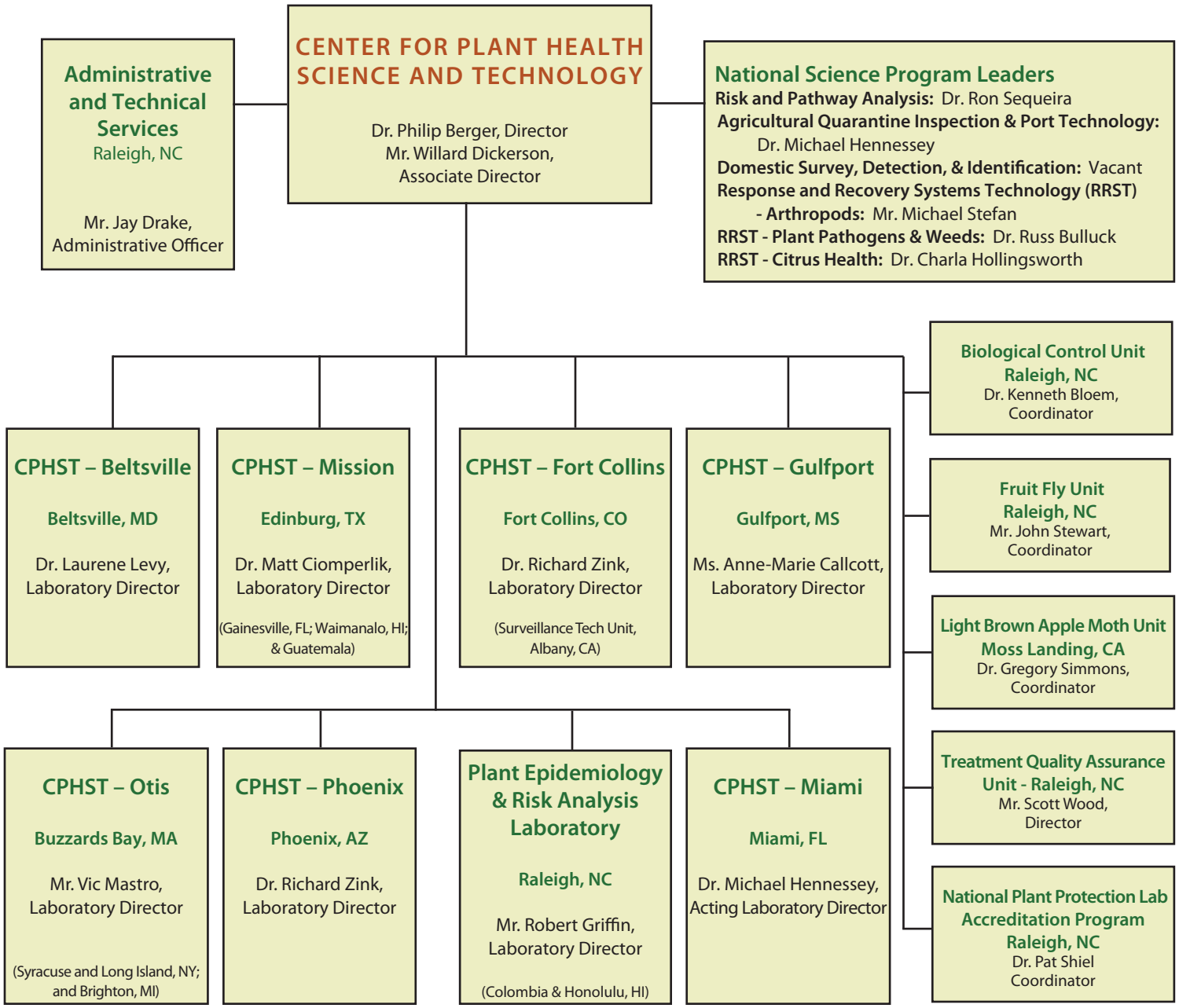

National Science Programs

Agricultural Quarantine Inspection and Port Technology

National Science Program Leader - Dr. Michael K. Hennessey

The Agricultural Quarantine Inspection and Port Technology (AQI&PT) program provides scientific support for agricultural inspection and commodity treatments to prevent the entry or spread of invasive quarantine pests. This program supports PPQ and the U.S. Department of Homeland Security's Customs and Border Protection (CBP) inspection and treatment programs. Requests for methods development are received from PPQ programs for port operations, trade, regional operations, and emergency pest programs, as well as CBP's agriculture inspection program. The AQI&PT program directly supports plant health officers and agriculture specialists stationed at 17 plant inspection stations (PIS) and over 350 cargo ports throughout the United States as well as numerous international ports that have preclearance programs.

Several CPHST laboratories work with this program, including Otis, Mission, Gulfport, Fort Collins, and the Plant Epidemiology and Risk Analysis Laboratory. A new agricultural quarantine inspection lab in Miami, FL, is currently being renovated and is expected to open in the fall of 2011. The Treatment Quality Assurance Unit (TQAU), a branch within the AQI&PT program, provides treatment certification activities and assists in transferring technology to the field. One major area of accomplishment in 2010 was emergency treatment validation of methyl bromide fumigations for new quarantine pests, including light brown apple moth (LBAM), European grapevine moth (EGVM), and spotted wing *Drosophila*. These treatments were needed to support the export of West Coast fruit shipments, but were also used to enable continuing Chilean fruit imports.

In 2010, AQI&PT also obtained a Technical Assistance for Specialty Crops Grant for Export of Irradiated Commodities. This grant focused on quality and logistical studies designed to encourage U.S. peach and blueberry exporters to utilize irradiation in order to meet other countries' quarantine requirements. In addition to PPQ funding, Farm Bill Section 10201 funding was obtained to support several projects.

Recent Accomplishments

Postharvest Treatments for EGVM. Validated a fumigation treatment for this pest for Chilean grapes.

New Knockdown Spray for the Japanese Beetle. Generated a list of candidate aerosol insecticides for replacement of d-phenothrin in cargo holds and airplane cabins, which will be evaluated for control of the Japanese beetle and other flying insects.

Treatments for Exotic Fruit Flies. Worked with international collaborators in Austria and Egypt on treatments for high-risk fruit flies, including *Bactrocera zonata*, *B. invadens*, and *Ceratitis capitata*.

Methyl Bromide Alternatives for Wood and Logs. Conducted trials of vacuum-steam heat treatments for wood pallets at Virginia Tech that showed this treatment can meet international standards. A larger trial using hardwood logs is planned for 2011.

Irradiation for Specialty Crop Exports. Completed studies to evaluate irradiation as a treatment for exported peaches and blueberries. Based on this study, U.S. growers are using this treatment on a pilot scale for peach exports to Mexico. This project was funded through a Technology Assistance for Specialty Crops grant for shipping and post-shipment quality assessment.

Plant Traceability. Completed trials on nursery plant traceability using barcodes and reader equipment at the National Arboretum. The trials provided insight for the next phase of testing at post-entry quarantine nurseries, including the need for equipment upgrades.

Isotope Analysis for Traceback. CBP completed stable isotope analysis of mangos from California, Florida, and Puerto Rico. Differences between isotope signatures from the three locations will be useful in determining the geographic origin of intercepted smuggled mangos.

Decontamination of Farm and Military Equipment. Conducted preliminary tests that showed chlorine dioxide was effective against bacterial spores. Additional tests will be conducted to determine efficacy for insect pests.

Sterilization and Disposal of Intercepted Quarantine Material. Demonstrated that commercial digester and chipper steamer equipment devitalized weed seeds.

Portable Gas Spectrometer for Detection of Invasive Pests. Initiated a 4-month trial of a portable gas spectrometer (Z-nose) at the Los Angeles PIS to evaluate its capacity to detect citrus cuttings.

International Treatment Support. The Colombia work unit conducted a risk assessment for *Copitarsia* moth species on flowers, determined the host status of cape gooseberries and passion fruit for exotic fruit flies, and developed a hot water treatment for fruit fly pests of passion fruit.

Packinghouse Procedures for Mitigation of Armored Scale. Developed a systems approach utilizing packinghouse procedures to mitigate armored scale on avocados from Mexico.

Dispersal of Armored Scale. Conducted studies to examine the dispersal of armored scale crawlers from discarded fruit to inform an updated pest risk analysis.

Citrus Leaf Wash Treatment. Developed a highly effective process for removing Asian citrus psyllid from kaffir lime leaves using a commercially available fruit and vegetable wash.

Quarantine Treatment for Hitchhiking Snails. Demonstrated that methyl bromide and sulfuryl fluoride fumigation was effective against snails in propagative plant materials, but also caused some damage to the plants.

Sampling Method for PIS. Designed a hyper-geometric sampling method to more effectively sample material during inspection. The sampling method will be implemented in 2011, and CPHST will be involved in training PIS inspectors.

Treatments for Mites on Citrus and Grapes. Initiated a cooperative agreement with the University of Florida to explore agricultural oils, soaps, and biopesticides as new treatments for *Brevipalpus* mites on fruit commodities as alternatives to methyl bromide fumigation.

Risk and Pathway Analysis

National Science Program Leader - Dr. Ron Sequeira

The Risk and Pathway Analysis (RPA) program focuses on collecting and interpreting scientific evidence and technical information regarding plant pest risks. RPA products help APHIS design risk-based policies and regulations for import, export, and domestic programs. The work of the RPA staff is essential to identify and assess new pest threats, provide scientific support for regulatory updates and revisions, and help prioritize resources to maximize plant health safeguarding capabilities.

The RPA program is supported primarily by CPHST's Plant Epidemiology and Risk Analysis Laboratory in Raleigh, NC, with satellite staff in Hawaii and Bogotá, Colombia. Additionally, RPA coordinates with TQAU, other CPHST laboratories, other APHIS and USDA groups, academia, foreign counterparts, and stakeholders to develop scientific analyses that are essential to producing risk-based phytosanitary policies and procedures. RPA program capabilities continue to be a central function for PPQ and an international benchmark for pest risk analysis.

The foundation of RPA's analytical capacity is its diverse, high-caliber scientific staff—which includes entomologists, plant pathologists, botanists, ecologists, economists, and other specialists—along with its extensive collection of information on plant pests. RPA uses state-of-the-art tools and methodologies for pest risk assessment. These include sophisticated spatial technology systems that integrate weather, pest distribution, and other databases to analyze pest dynamics; identify pests of greatest concern; identify potential pathways for the introduction of harmful exotic pests; and predict the spread of exotic pests.

In 2010, the RPA program continued to lead APHIS in the development of analytical products to support emergency, domestic, and trade programs. In addition, the RPA program addressed high-profile trade issues linked to commodity importations and produced commodity risk analyses that supported free trade agreements. In 2010, the RPA program finalized more than a dozen commodity import requests and completed several key risk assessments and pathway analyses. Critical products included those that supported regulations for citrus diseases. Mission-critical rules or analyses included

those for citrus canker, citrus nursery stock, sweet orange scab (SOS), citrus black spot (CBS), LBAM, and several "routine" commodity specific risk/pathway analyses.

Future plans include promoting the continued evolution of risk analysis tools and methodologies while also increasing the transparency and efficiency of risk analysis work processes. To address climate change as one of USDA's new policy objectives, we plan to incorporate climate change scenarios into current risk analysis products.

Recent Accomplishments

Pathway Research on Mites of Quarantine Importance. Coordinated a cooperative agreement with the University of Florida to conduct field and laboratory studies on the likelihood of dispersion and establishment of *Brevipalpus* quarantine mites that are potential vectors of Citrus leprosis. The results of this research advanced the knowledge on the risk of mites spreading to host plants through an imported fruit pathway.

Exploring New Mite Treatments. Coordinated with the AQI&PT program and the University of Florida to test agricultural oils, soaps, and biopesticides on regulated *Brevipalpus* pest mites found on fruit commodities. The purpose of these studies was to determine the potential use of these low toxicity products as alternatives to methyl bromide fumigation for regulatory applications.

CBS Management Recommendations. Coordinated the development of recommendations for different potential scenarios related to CBS in Florida so that PPQ is prepared with science-based recommendations if new finds occur.

CBS Risk Assessment. Coordinated the risk assessment update for CBS to ensure a scientific basis for developing regulatory activities. An internal and external review of the risk assessment was conducted. The updated APHIS risk assessment document was used to inform decisionmaking. Updates to the current regulatory requirements may save U.S. growers millions of dollars due to facilitated domestic and international trade.

SOS Pathway Analysis. Coordinated the completion of a critical pathway analysis for SOS to assess the likelihood that citrus fruit may spread the disease. This analysis was used to inform APHIS' decisionmaking for a Federal Order establishing SOS quarantine areas. The analysis determined that fresh fruit is not an epidemiologically significant pathway for the spread of SOS, provided that it undergoes industry-based packinghouse procedures. Interstate sales of citrus from quarantined areas are allowed if these procedures are used, which will prevent spread of the disease to other States while avoiding economic damage to the citrus industry in the quarantined areas.

Asian Citrus Psyllid (ACP) and Huanglongbing (HLB) Field Research and Outreach Project. Coordinated Farm Bill-funded cooperative program projects with the North Carolina State University's Center for Integrated Pest Management and Chinese institutes. The objective of the project was to collect information on ACP-HLB management systems in China to inform U.S. citrus producers. With these projects, RPA:

- Completed an information system for Chinese literature collection on the subject of HLB and ACP. More than 700 references have been incorporated into the system with translations of key information.
- Reviewed citrus production practices in China and reported that commercial citrus groves will survive for many years after the appearance of citrus greening disease symptoms with optimal irrigation and fertilization management.
- Reported on Chinese studies that focus on nutritional approaches to the management of citrus greening.
- Obtained a new biological control resource, which included a comprehensive list and taxonomic description of the entire family of coccinellids (ladybeetles) in China.
- Identified three new organisms that are potential biological control agents for ACP. One strain of a *Beauveria* fungal pathogen on ACP causes more than 90-percent mortality of nymphs and adults.

Soaps and Oils Against ACP. Coordinated a Farm Bill-sponsored study of the use of soaps and oils against ACP. The study showed that some soap formulations were 100-percent effective as contact insecticides against the psyllid, and that different oils vary in their effectiveness in repelling ACP. These findings are critically important for citrus greening disease management in organic groves, nurseries, and greenhouses.

Climate Change Plan. Began establishing a Climate Change Plan for PPQ in 2010. This effort was expanded to all groups within APHIS, with leadership provided by CPHST. A draft document has been developed for vetting

by APHIS leadership.

Domestic Survey, Detection, and Identification

National Science Program Leader - Vacant

The Domestic Survey, Detection, and Identification (SDI) program provides scientific and technical support to PPQ for rapid detection, identification, and mitigation of plant pests. SDI consists of three cross-functional groups: early detection, identification, and spatial technology. CPHST scientists work as teams to address the scientific and technical needs of PPQ leadership and operations. Additional support is leveraged through cooperation with academic institutions, industry, and other government agencies.

The early detection team supports pest surveillance through the PPQ Cooperative Agricultural Pest Survey (CAPS) program. Support includes pest prioritization, pest datasheets and survey guidelines, approved methods for the survey and identification of CAPS targets, and trap and lure development. Each year, CPHST develops an updated, prioritized list of pests for the CAPS program. This list consists of exotic, high-impact pests that threaten U.S. agriculture and the environment. In addition, CPHST works with the CAPS program to develop pest lists for commodities and taxon groups recognized as national CAPS targets. Surveys focused on specific crops or types of pests provide an efficient method for detecting a suite of exotic pests at the same time. CPHST provides survey and identification support for pests from both the prioritized pest list and national surveys.

In 2010, SDI scientists developed CAPS-approved survey and identification/diagnostic methods for each of the 119 CAPS priority pests. In addition, CPHST developed pest datasheets for new CAPS priority pests, revised existing survey protocols, and produced new commodity-based survey documents. These resources are used by the States when planning and conducting surveys. CPHST also works with scientists from universities and other government agencies to develop and improve traps and lures for CAPS targets.

The Identification Technology Program (ITP) team delivers innovative, client-friendly, digital diagnostic aids, tools, and resources to PPQ and cooperators. These tools support identification responsibilities associated with pests, diseases, and weeds. In 2010, ITP delivered five Internet-based tools, including *A Resource for Pests and Diseases of Cultivated Palms*, the team's first commodity-based suite of tools to support domestic survey and detection. ITP also released ID Source, which is a gateway for Internet-based identification resources for plant pests and provides links to factsheets, screening aids, and image galleries.

The spatial technologies team integrates data collection, management, analysis, statistics, and modeling to support PPQ's broad needs. With members located in CPHST headquarters and laboratories across the country, the team contributes to a wide range of programs, including pest surveillance, monitoring, and control, as well as supports emergency programs, global risk assessments, and safeguarding operations.

In 2010, the team contributed to numerous plant health programs. Highlights include the completion of an introduction risk model for the Asian gypsy moth in California. This model built upon previous models developed for Washington and Oregon and finalized a predictive tool, which is defined by expertise from the field and supported by those directly involved in trapping program management. Also, the team has continued work on the ACP areawide monitoring study for the Lower Rio Grande Valley in Texas in an effort to better understand ACP dynamics and the spread of citrus greening disease. In addition, the team supported department-level directives on climate change, supported CPHST scientists using the global positioning system (GPS) for invasive weed monitoring, and further contributed to analyzing insect population dynamics for grasshoppers in the Western United States.

The spatial technologies team also supported and developed advanced tools for the North Carolina State University/APHIS Plant Pest Forecasting System (NAPPFAST, www.nappfast.org), which can be used to create models and maps for plant pests. This system supports CAPS and emergency programs, Internet-based decision support, and establishment modeling. In 2010, two new NAPPFAST risk map types were generated. The first type incorporates pest interception data, the Freight Analysis Framework, and pest association with specific commodities. The second type uses a statistical algorithm to combine multiple risk maps to produce a more descriptive risk map. Currently, the spatial technologies team is developing a zonal statistics pest ranking tool to assist and streamline the risk comparison process for various Federal and State cooperators.

Recent Accomplishments

Early Detection Team Support for CAPS

- Completed the Corn Commodity-Based Survey Reference and Guidelines.
- Revised the Exotic Wood Borer/Bark Beetle National Survey Guidelines and added datasheets for 11 new pests.
- Developed six pest datasheets for pests on the CAPS Prioritized Pest List.
- Developed approved survey and identification/diagnostic methods for each of the 119 CAPS priority pests.

Identification Technology Program Releases

- *Federal Noxious Weed Disseminules of the U.S., Edition 2.1*, http://keys.lucidcentral.org/keys/v3/FNWE2/
- *An Identification Tool for Bark Beetles of the Southeastern United States*, http://keys.lucidcentral.org/keys/v3/Bark_Beetles/
- *A Resource for Wood-Boring Beetles of the World: Xyleborini Ambrosia Beetles – An Identification Tool to the World Genera*, http://itp.lucidcentral.org/id/wbb/xyleborini/index.htm
- *A Resource for Pests and Diseases of Cultivated Palms*, http://itp.lucidcentral.org/id/palms/resource/index.html
- The beta version of ID Source, available at http://idsource.colostate.edu

Spatial Technologies Team Products

- Updated all NAPPFAST maps for over 230 CAPS targets with new host information and included Alaska, Hawaii, and the territory of Puerto Rico.
- Generated new NAPPFAST risk map types using interception data, freight analysis, and by combining multiple risk maps.
- Delivered the Asian Gypsy Moth Trapping Model for California to stakeholders for the 2010 field season.
- Finalized and delivered invasive weed GPS monitoring data to cooperators for 10 weeds in 7 States.
- Continued work to analyze ACP and grasshopper population dynamics.

Response and Recovery Systems Technology - Arthropods

National Science Program Leader - Mike Stefan

The Response and Recovery Systems Technology (RRST) program for arthropod pests provides timely scientific support to regulatory program managers and decisionmakers for high-consequence plant pests. The program emphasizes science-based preventive measures to reduce or inhibit further spread and develops rapid response and recovery technologies geared towards eradicating or containing targeted pests of concern. RRST supports a wide range of programs and oversees the CPHST LBAM Unit and the Fruit Fly Unit. These programs utilize the tools of current integrated pest management strategies, phytosanitary measures, regulatory treatments, and related technologies to mitigate the effects of arthropod pests while minimizing adverse effects on the environment, producers, and consumers.

Recent Accomplishments

EGVM, *Lobesia botrana*

- Survey activity during 2010 determined that the moth was present in several grape-producing counties in California.
- A CPHST-led technical working group (TWG) provided information and feedback on issues regarding trapping, lures, mating disruption, treatment, hosts, risks associated with commodities, and treatment of regulated articles.
- During the 2010 growing season in California, based on trapping and phenology information, the predictive emergence of each generation was utilized to time treatments as part of an areawide control program to reduce populations and to limit spread.
- The TWG met in the fall of 2010 to review the activities and results of the program in order to provide recommendations for the next phase.
- Survey, areawide treatment, and research activities will continue during the course of the 2011 growing season.

LBAM, *Epiphyas postvittana*

- CPHST continued to chair a TWG of scientists from the United States, Australia, and New Zealand to consider potential eradication/control options, develop management recommendations, assess research needs, and provide ongoing scientific input to program operations.
- Four new rearing units were installed at the LBAM Unit rearing facility in California in 2010, significantly increasing the sterile insect production capacity. CPHST established the LBAM Unit in 2009 to develop sterile insect release technology for the moth and support the program's control and management goals.
- The LBAM Unit has continued to support regulatory treatment testing to allow movement of commodities from regulated areas.

- The CPHST Otis Lab conducted cold hardiness studies for LBAM through a cooperative agreement with the University of Minnesota, providing the program with data to base risk assessments for survival and establishment in northern States.
- Through a cooperative agreement with the University of California, research on the phenology of LBAM in California has provided information on potential biocontrol agents, the potential for distribution of LBAM in North America, and lifecycle vulnerabilities for management options.

Fruit Fly Introductions
- Began working in cooperation with the Fruit Fly Coordinator, a newly created CPHST position, in 2010.
- Provided scientific support to eradication efforts for Mediterranean fruit fly (Medfly) in California and Florida, Mexican fruit fly (Mexfly) outbreaks in California, and the Mexfly eradication program in Texas.
- Provided support and guidance for the labs associated with the Gradual Advance Plan in Guatemala.
- Supported genetic barcoding studies for enhanced identification data of Oriental fruit fly and other *Bactrocera* species outbreaks.
- Initiated a pilot project with the Fruit Fly Coordinator to identify and barcode *Anastrepha* fruit flies from intercepted baggage from two U.S. ports to identify the point of origin using molecular genetics. In addition, tested protocols and procedures for the collection of quality specimens for barcoding.

Emerald Ash Borer (EAB), *Agrilus planipennis*
- Continued to support research on improving traps, lures, control, and management of EAB.
- Supported a mass-rearing operation in Michigan in order to facilitate widespread field releases of identified parasitoids.

Asian Longhorned Beetle (ALB), *Anoplophora glabripennis*
- Continued to support the PPQ quarantine, control, and eradication programs for ALB in Massachusetts, New York, and New Jersey.
- Coordinated research on attractant lures and trapping research with university cooperators and USDA's Agricultural Research Service in Massachusetts and New York.
- Developed a cooperative agreement with the PPQ National Detector Dog Training Center for a pilot program to test the possibility of using detector dogs in ALB surveys.
- The CPHST Otis lab continues to provide technical support to the ALB program through testing of regulatory treatments, control treatments, and discerning infestation dynamics.

***Sirex* Woodwasp (*Sirex noctilio*) Biological Control**
- Supported the research of a CPHST scientist in Syracuse, NY, on host preference, attractant volatiles, and trap studies.
- Scientists from the CPHST Otis Lab conducted controlled release experiments of the nematode biocontrol agent, *Beddingia siricidicola*, and investigated the native strain of *B. siricidicola*.
- The CPHST-led TWG met in January 2010 to discuss pest status and spread, survey, control, and research needs.
- Working together with USDA's Forest Service, continued to develop survey tools and evaluate the effectiveness of the biological control agent.

Kudzu Bug, Bean Plataspid Bug, *Megacopta cribraria*
- Developed a cooperative agreement with the North Carolina Department of Agriculture to determine host preference, including soybean varieties.

Emergency Response and Response and Recovery Systems Technology - Plant Pathogens and Weeds

National Science Program Leader - Dr. Russ Bulluck

In 2010, the responsibilities for the Emergency Response and Response and Recovery Systems Technology (RRST)-Plant Pathogens and Weeds programs were combined under the leadership of Dr. Russ Bulluck. The Emergency Response program's role is to provide regulatory officials at PPQ headquarters and in the regions with scientifically valid and operationally practical information to be used during a plant health emergency. This information is usually related to the biology of the plant pest and includes information related to the identification of the organism, surveillance methods, and management methods. During the initial plant health emergency response, a TWG of scientific experts may also be assembled to provide the necessary background information.

The Emergency Response Program Leader is responsible for facilitating and coordinating the interactions of the appropri-

ate Response and Recovery programs and working in concert with these program leaders to ensure a continuum of scientific support during transitions from emergency response to recovery. Numerous plant health emergencies have occurred this year, including outbreaks of EGVM, Medfly, CBS, SOS, and several others. In fact, during the past year, approximately 18 different plant health emergencies required scientific input.

Another important part of the Emergency Response program is preparing for emergencies. The program works closely with PPQ's Emergency and Domestic Programs (EDP) section. CPHST collaborates with EDP to prepare New Pest Response Guidelines (NPRG) for new and emerging pests, which are to be used in the event that the exotic pest is detected within the United States. CPHST provides scientific information on the biology of the organism, control methods, survey methods, ecology of the pest, and identification methods. Recent NPRG include: *Phytophthora* species in Forest and Nursery Systems, Red Palm Weevil, Citrus Variegated Chlorosis, Citrus Leprosis, and Cotton Seed Bug.

By working in concert with other national programs in CPHST and programs within PPQ, the Emergency Response program is able to add value to many different areas with scientifically valid and operationally practical information on plant health emergencies.

Recent Accomplishments

National Ornamental Research Site at Dominican University of California (NORS-DUC). The NORS-DUC is a secure site that simulates a nursery and is used to perform pest and disease studies on nursery stock. These studies are based on the epidemiology and behavior of pathogens and pests in a "real world" environment and ensure high-level safeguards to prevent the escape of pathogens and pests. Research scientists from the national and international community have submitted proposals for work at this site on the pathogen *Phytophthora ramorum*, the cause of ramorum blight on ornamental plants in nurseries and sudden oak death in California (with a small outbreak in Oregon). There are currently five research projects underway with an additional three to four planned in 2011. There are currently two different phases of NORS-DUC in operation. The first phase located in Forest Meadows has eight bays, with all but one occupied with research projects. The second phase was completed in December 2010 with an additional five bays for research.

Potato Cyst Nematode Programs—Pale Cyst Nematode in Idaho and Golden Nematode in New York. These programs have been in place since the early 1970s in New York and since 2006 in Idaho. CPHST continues to provide scientific support for both programs. CPHST provides scientific information related to the biology and control of these nematodes as well as information regarding sampling strategies and protocols.

NPRG Scientific Team. NPRG provide basic information on the biology of specific pests or groups of pests. These documents are most useful prior to a plant health emergency involving a pest. The Farm Bill of 2008 specifically targeted the development of "action plans" to combat new plant and animal health emergencies. NPRG provide these plans and include information on organism biology, survey and control methodologies, and environmental and regulatory components supplied by EDP. Through Farm Bill funding, four post-doctoral fellows were hired to work specifically on NPRG. To date, 10 NPRG have been completed or are in the process of being completed. In some emergencies, a pest is detected prior to the development of NPRG. In 2010, this occurred for CBS and SOS. These NPRG were expedited so that information on the biology, survey, controls, and other relevant issues could be provided in a timely manner.

Management of TWGs. Often when new plant health pests and pathogens occur within the United States, very little information is readily available on the specific organism. This is due, in large part, to the fact that the pests and pathogens are almost always exotic and any scientific expertise resides offshore. One of the methods used to obtain this scientific information is through the use of TWGs. TWGs bring together scientific experts from around the world and can be accomplished either through full face-to-face meetings or by video or telephone conferences. During the past year, we were involved in approximately 10 different TWGs ranging from full face-to-face meetings (in conjunction with other meetings) to email-based communications in which a series of questions was sent out to more than 100 participants. For example, an SOS TWG was held at the American Phytopathological Society meeting, which saved more than $10,000 in travel costs for five Federal and six non-Federal TWG members. The program compiles and reports the results from TWG meetings with input from the PPQ national program that requested the TWG. The report provides

specific recommendations to the PPQ program based on the science of the plant health pest.

Planning and Preparing for Plant Health Emergencies. One of the Emergency Response program's major functions is to help APHIS prepare for plant health emergencies. The program is involved to varying degrees in the planning and preparation of Plant Health Emergency exercises as well as exercise participation. These exercises range from phone call and email chains to tabletop exercises to full-scale Incident Command System exercises and Area Command exercises. Last year, the program helped plan and facilitate eight separate full-scale and tabletop exercises. Some of the staff was involved with direct planning of the exercises, while others took part in the retrieval of scientific information. By separating the planners from the responders, staff members were also able to participate in the exercise as both exercise facilitators and participants to practice the response during an actual plant health emergency.

Response and Recovery Systems Technology - Citrus Health

National Science Program Leader - Dr. Charla Hollingsworth

In 2010, some of the responsibilities for the RRST-Plant Pathogens and Weeds program transitioned from Dr. Charla Hollingsworth to Dr. Russ Bulluck. Dr. Hollingsworth's responsibilities shifted to focus exclusively on citrus health because of the critical need for a citrus health coordinator due to the introduction of numerous citrus diseases over the last few years. The National Science Program for RRST-Citrus Health is responsible for supporting methods development work to prevent the introduction and establishment of citrus pathogens of regulatory concern in the United States. The program is currently working on previously established plant diseases, such as HLB and citrus canker, as well as newly emerging diseases, such as SOS and CBS. The program emphasizes an integrated, multifaceted approach for controlling plant disease through discovery and implementation of effective best management practices. Working cooperatively with others in CPHST and elsewhere, RRST-Citrus Health supports the development of applied, basic, and technological tools to accomplish effective response and recovery while preserving the integrity of the production environment.

Working closely with the Citrus Health Response Program (CHRP) to preserve citrus health in the United States is a primary mission of the program. This is accomplished through interactions with researchers, technical experts, regulatory program managers, stakeholders, and others. During 2010, numerous TWGs were established to address disease issues of citrus. Specifically, TWGs addressed uniform national survey standards for ACP and HLB, areawide control strategies for ACP, as well as response and recovery efforts for CBS and SOS.

In addition to other responsibilities, RRST-Citrus Health assumed a leadership role regarding the National CHRP Science and Technology Coordination Group. In this role, the program coordinates citrus research efforts nationally and internationally, develops and maintains a database of citrus-related research within the United States, and organizes an annual meeting that brings together researchers, technical experts, and regulatory programs from across the United States. This effort supports open communication and research coordination across the citrus research community to benefit scientific inquiry and inform stakeholders.

Recent Accomplishments

- Developed national surveillance methods and strategies for ACP and HLB. Strategies were developed to encompass unique location circumstances found throughout U.S. citrus production areas: (1) when ACP and HLB are present, conduct an areawide insect and disease assessment survey; (2) if ACP is established and HLB is not reported, conduct insect abundance and disease detection surveys; and (3) if ACP and HLB are not established, conduct a detection survey.
- Developed and delivered an inspection strategy for asymptomatic fruit from SOS-infested areas to the CHRP. This strategy design allows asymptomatic fruit from SOS quarantined areas to move to citrus-producing areas.
- Developed and delivered survey and treatment strategy for nursery stock from SOS-infested areas to the CHRP. These protocols are designed to provide adequate safeguards for the safe movement of ornamental citrus nursery stock to non-citrus-producing States.
- Developed and delivered detection and delimitation survey methods for SOS to the CHRP. Three survey strategies were designated as: (1) random detection—general survey of citrus production areas; (2) targeted detection—survey for use in citrus nursery propagation facilities; and (3) delimitation—survey to define the scale and scope of an infestation.

- Developed and delivered an SOS field identification guide to agriculture surveyors. This pictorial guide provides high-quality pictures of confirmed SOS symptoms as well as diseases with similar symptoms that could present confusion.
- Developed and delivered a soil and water sampling protocol to the *P. ramorum* National Program. This protocol replaces the previous versions and has been incorporated into the Confirmed Nursery Protocol for *P. ramorum*.
- Completed a review of extant literature related to soil and water sampling methods for *P. ramorum*.
- Compiled a database of *P. ramorum* literature encompassing soil and water sampling methods.
- Taught attendees of two water and soil survey training workshops about *P. ramorum*. Attendees consisted of PPQ and State department of agriculture officials.

Laboratories and Units

CPHST Lab, Beltsville, MD

Laboratory Director - Dr. Laurene Levy

The Beltsville Lab's mission is to develop, validate, and implement advanced biochemical and molecular methods for the detection of high-consequence plant pathogens, including APHIS select agents and plant pathogens in foreign germplasm. Laboratory programs utilize cutting-edge technologies from the fields of plant pathology, molecular biology, and clinical diagnostics to develop, adapt, and improve methods for accurate and rapid diagnosis of plant pathogens. The laboratory's scientists validate plant pathogen diagnostic methods prior to stakeholder release to assure their performance and fit for purpose in regulatory programs.

The laboratory strives to achieve timely transfer of diagnostic tools that are field deployable for PPQ emergency response and eradication programs. Tools are deployed to stakeholders through clearly written standard operating procedures and hands-on laboratory training for end users within and outside of PPQ. The laboratory also utilizes new diagnostic methods to accurately and rapidly diagnose and differentiate high-consequence and select agent plant pathogens, or pathogens that require Federal confirmation. In these situations, the laboratory conducts sample testing in parallel with the PPQ Molecular Diagnostics Laboratory. The Beltsville Lab is enhancing its forensics capabilities to understand where the pathogens originate or are emerging. This effort will support mitigation efforts by aiding in the determination of pathogen incursion pathways.

Recent Accomplishments

- Completed serological characterization of the *Plum pox virus* (PPV) strain W isolate 44189 intercepted from the Ukraine. Improved a strain W-specific polymerase chain reaction (PCR) assay to efficiently amplify more PPV W isolates, specifically those from the Ukraine.
- Cellular Analysis and Notification of Antigen Risk and Yield (CANARY) is a cell-based technology that is capable of rapidly identifying low levels of plant pathogens. We demonstrated that the CANARY assay was superior in detection sensitivity compared to standard immunestrip assays for R*alstonia solanacearum*. We also developed protocols and procedures for a *Phytophthora* and a potyvirus CANARY.
- Established a CANARY cell line for citrus variegated chlorosis for evaluation in FY 2011.
- Validated conventional and quantitative PCR assays for detection and diagnosis of *Guignardia citrocarpa*, the pathogen that causes citrus black spot (CBS). Completed and released work instructions for these protocols.
- Validated conventional PCR assays and developed a real-time PCR assay for detection and diagnosis of *Elsinoë australis*, the pathogen that causes sweet orange scab (SOS). Completed and released work instructions for these protocols.
- Validated PCR assays for detection and diagnosis of *P. kernoviae* for release in FY 2011.
- Developed and released a proficiency test panel for PPV in June 2010.
- Distributed the 2010 *P. ramorum* proficiency test. Based on this test, 28 diagnosticians from 16 laboratories were certified to perform *P. ramorum* diagnostics for PPQ.
- Released the 2009 Huanglongbing (HLB) proficiency test. Sixteen diagnosticians were evaluated and certified in FY 2010 to perform HLB diagnostics for PPQ.
- Developed and adapted 17 conventional and real-time PCR assays for specific viruses in foreign germplasm. Assays were released for *Potato black ringspot virus*, *Tomato ringspot virus*, *Tomato black ring virus*, *Sweet potato latent virus*, *Sweet potato virus 2*, *Arabis mosaic virus*, *Strawberry latent ringspot virus*, *Cocksfoot streak virus*, *Johnson grass mosaic virus*, and *Sugarcane mosaic virus*. In addition, assays were developed for PCR detection of potyviruses infecting grasses and sweet potato.
- In June 2010, three Beltsville Lab scientists traveled to Charlotte, NC, as part of an Eastern Region/CPHST coordinated team that performed molecular diagnostics within the PPQ/Agricultural Research Service (ARS) containment mobile laboratory/greenhouse. In this field setting, the team completed diagnoses on over 150 plant samples from a chronically *P. ramorum*-infested site. The activity identified three *P. ramorum*-positive samples.
- Delivered eight hands-on training courses in FY 2010, including:
  - Molecular Detection of Huanglongbing in Plants and Psyllids (November 30–December 4, 2009, and April 27–29, 2010);
  - *Phytophthora ramorum* 101 (April 13–16, 2010);
  - Detection of *Plum Pox Virus* (April 20–22, 2010);
  - Introduction to Bio-Informatics for Diagnosticians (July 12–14, 2010; August 23–25, 2010; August 25–27, 2010); and
  - Detection of *Ralstonia solanacearum* (November 8–10, 2010).

CPHST Lab, Fort Collins, CO

Laboratory Director - Dr. Richard Zink

The Fort Collins Lab develops and transfers scientifically based methods and innovative tools to APHIS and other Federal and State agencies to reduce risk levels associated with plant pests and plants for planting. In addition, the laboratory provides scientific support for pest detection and survey methods under the Cooperative Agricultural Pest Survey (CAPS) program. The laboratory transfers methodologies and tools to field operations, ensuring efficient and effective survey, detection, identification, emergency response, and eradication. The laboratory also develops electronic, matrix-based identification resources to help support rapid, consistent, and accurate identification of pest species.

The Fort Collins Lab develops management methods for invasive plants and plants on the Federal Noxious Weeds list through applications of biological, chemical, and/or cultural controls. 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 laboratory in Albany, CA, we continue to improve upon artificial diets and other control tactics for the control of light brown apple moth (LBAM) and the rearing of biological control insects for two noxious weeds, purple loosestrife (*Lythrum salicaria*), and the knapweed complex (*Centaurea* spp.).

Recent Accomplishments

- Developed laboratory and greenhouse methods for the evaluation of plants that are potential invasive weeds to support Federal regulation. Developed molecular diagnostic tools for invasive plants.
- Developed a geospatial model that predicts areas of highest Asian gypsy moth (AGM) introduction risk for the Western United States, including the States of Washington, Oregon, and California. The model is being used by PPQ, State departments of agriculture, and the U.S. Department of Homeland Security (DHS).
- Delivered six electronic identification tools in 2010 and managed 34 identification products in cooperation with 18 institutions. In sheer numbers, 2010 saw more identification products in development and delivered than in any prior year.
- Tested waste disposal machines that are currently used by hospitals and research labs for use in disposal of regulated airline waste and found that these machines would be a more cost-effective alternative. The cost of regulated airline waste incineration at international airports was about $2,600 per ton in 2009 when handled by a waste disposal vendor.
- Conducted prerelease research on nine weed targets: Canada thistle, dyer's woad, garlic mustard, hawkweeds, hoary cress, hound's-tongue, perennial pepperweed, Russian knapweed, and yellow toadflax.
- Facilitated field releases of Russian knapweed and yellow toadflax biocontrol agents in several western States.
- Improved artificial diets for three potential biocontrol insects (one each on loosestrife, the diffuse/spotted knapweed complex, and LBAM).

CPHST Lab, Gulfport, MS

Laboratory Director - Anne-Marie Callcott

The CPHST Gulfport Lab continues to support PPQ programs through the analytical chemistry section and the imported fire ant (IFA) section. The analytical chemistry section primarily processes APHIS pesticide treatment program samples and provides technical support in the form of methods development to address changing program needs. In 2010, the program sample loads increased about 1.5-fold over recent sampling trends between 2006 and 2009. In addition to the routine work surge, the chemistry methods development staff shifted resources from routine methods adaptation work to more specialized work supporting the development and verification of lures used by PPQ programs and by CPHST scientists. This work directly supports the increased need for quality assurance testing of lures purchased by PPQ for CAPS survey and provided analytical support for PPQ survey, control, or research programs utilizing lures in 2010.

The IFA section develops methods and tools for the survey, detection, regulation, and chemical and biological control of the IFA. Technology developed by the IFA section is utilized by PPQ, State plant regulatory officials, the nursery industry, the chemical industry, farmers, homeowners, and other stakeholders. The primary focus is on the development of quarantine treatment options for growers who move regulated articles, such as grass sod and field-grown nursery stock, outside of the Federal quarantine area.

Recent Accomplishments

- Analyzed 1,230 APHIS routine program-related samples, including environmental monitoring samples and lures along with 390 associated quality control samples.
- Analyzed 1,400 samples supporting projects requested by other APHIS scientists and program leaders.
- The analytical chemistry staff was recognized by the APHIS grasshopper program with a group award for the accurate, swift, and reliable analyses of environmental monitoring samples associated with 2010 program treatments, including urgently processing and developing analytical methods for spill-site samples.
- In support of the APHIS grasshopper program, an improved analytical extraction method was developed and validated for determining diflubenzuron in vegetation. The new extraction method was significant in that it improved both detection through better chromatography (provided a cleaner extract for instrumental analysis) and reduced solvent use and waste in the extraction phase. This resulted in a lower limit of detection and quantification while reducing solvents associated with extraction by 95 percent (see Project Highlights for details).
- In support of the Asian longhorned beetle (ALB) program, a general method for extraction and analysis of imidacloprid in honey was quickly and successfully developed, validated, and applied to samples. The method is significant in that only 12 milliliters of organic waste is generated in the extraction phase of the method.
- The APHIS-funded IFA Phorid Fly (*Pseudacteon* species) rearing and release program continued in 2010 with multiple releases of a third fly species, and production rearing of a fourth species was initiated. A journal article on the establishment and spread success of the first two species, *P. tricuspis* and *P. curvatus*, was accepted for publication by the Journal of Insect Science (Volume 11, Article 19). Both biological control species are established in more than 50 percent of the IFA quarantined area.
- Data collected over several years, in collaboration with Tennessee State University's Nursery Crop Research Station, has led to a new application technique for a more effective IFA quarantine drench treatment of balled-and-burlapped nursery stock and a new insecticide for this use pattern. This new application technique of flipping the root ball between drench applications will significantly shorten treatment time (from 3 days to 1 day) for growers.

CPHST Lab, Miami, FL

Laboratory Director - Vacant

In response to the dynamic port, trade, and safeguarding environment that is associated with some of the highest levels of risk for plant and animal health, APHIS is in the process of establishing a state-of-the-art Agricultural Quarantine Inspection and Port Technology (AQI&PT) Development Laboratory that will provide leadership in agriculture quarantine inspection (AQI) treatment technology. The laboratory will be colocated with ARS at the Subtropical Horticulture Research Station. Renovation of the laboratory space is currently underway, and the lab is projected to open in fall 2011.

Vision

The vision is to provide world leadership in the development of phytosanitary measures and port technologies to protect American agriculture and natural environments from invasive alien species.

The AQI&PT Lab will develop scientific expertise regarding pest exclusion, design risk management systems, develop international AQI information networks, develop quarantine treatments, provide technical support for the treatment manual, develop methyl bromide alternatives, develop inspection methods for PPQ and DHS Customs Agricultural Inspectors, and support validation and certification for domestic and foreign treatment facilities in preclearance and domestic programs.

CPHST Lab, Mission, TX

Laboratory Director - Dr. Matt Ciomperlik

The Mission Lab consists of a multidisciplinary team of scientists and technical support staff with diverse knowledge and experience in developing management and mitigation strategies against plant pests, diseases, and noxious weed species. The Mission Lab plays a central role in developing molecular diagnostic tools for arthropod and invertebrate pests in support of PPQ programs. Other important roles include support for remote sensing/global information systems; epidemiology of plant diseases, such as citrus canker and SOS; fruit fly trapping; sterile insect technique (SIT) support for Mexican fruit fly (Mexfly) control; arthropod quarantine facilities to support biological control; and areawide/integrated pest management.

The molecular diagnostics unit focuses on the development and application of molecular diagnostic tools for pest arthropods, including fruit flies and other invertebrate pests such as gastropods. The unit has released DNA-based tools for identification of immature LBAM, developed new DNA procedures for analysis of the Mediterranean fruit fly (Medfly), and evaluated introduction pathways of LBAM, ALB, and fruit fly pests. Recently, the unit has been developing and evaluating a new technology called DNA barcoding for identification of numerous pest species, including moths, fruit flies, weevils, wasps, snails, and slugs.

The Arthropod Quarantine facility continues to support several PPQ programs through secure receipt, handling, rearing, and permitted release of numerous biological control agents. During FY 2010, the facility received multiple shipments of natural enemies in support of the Asian citrus psyllid (ACP), Russian knapweed, and giant cane (*Arundo*) biological control programs and maintained a wild colony of Mexfly in support of the eradication program. The quarantine facilities operations were formally documented in the Mission Lab's Quality Management System in 2010 and brought into the scope of the lab's International Organization for Standardization (ISO) registration.

Recent Accomplishments

- Completed studies on the efficacy of packinghouse procedures on the mitigation of armored scales on Hass avocado being imported from Mexico, adding valuable information for consideration in the revision of the risk assessment.
- In partnership with PPQ's Western Region and Texas A&M University-Kingsville's (TAMUK) Citrus Center, prescreened approximately 3,500 leaf samples for HLB/citrus greening disease in south Texas. TAMUK's PCR analysis of over 1,000 samples indicates that the Lower Rio Grande Valley remains HLB free.
- Completed development of a real-time PCR assay for identification of LBAM that reduces the cost and service time of moth diagnosis in California. This technology has been released as a work instruction for use by USDA and the California Department of Food and Agriculture.
- Identified genetic differences between a parasitic strain of *Beddingia siricidicola*, a nematode used in biocontrol of the *Sirex* woodwasp, and populations already present in North America that exhibit low rates of parasitism. This finding allowed for experimental release of the parasitic strain of the nematode.
- Revised a Medfly pathway analysis tool with 18 novel genotypes identified from 4 SIT facilities and 16 recently sampled wild fly populations. Assisted the Florida Department of Agriculture in genetic analysis of Medflies captured in Boca Raton, FL.
- Generated DNA barcode databases to identify fruit fly pests. The barcode information has been analyzed for several important pests, including the Medfly and Mexfly.
- Completed a 4,700-acre areawide pest management pilot project that demonstrated the efficacy of dormant season insecticide applications for controlling ACP. The timing and application methods have been implemented in a voluntary grower-driven program across the citrus-growing counties of the lower Rio Grande Valley of Texas.
- Conducted numerous tests for Mexfly diet development and rearing support, including: diet tests to maximize the quantity of high-quality flies while minimizing production costs; mating tests of a Willacy County strain against wild flies; validation tests on the use of Guatemalan bubbled eggs; tests on the effect of extended chilling on adult flies; and evaluation tests on aerial fly releases from single- and double-box release machines. Additionally, field studies for the eradication effort were conducted to evaluate fruit fly trap/lure/trapping agent combinations for possible use of bait stations in dooryards.

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

Laboratory Director - Vic Mastro

The Otis Lab's mission is to identify, develop, and transfer technology for survey, exclusion, control, and risk assessment for APHIS and its cooperators. The lab serves a wide variety of PPQ programs that include: exotic pest-detection programs, phytosanitary treatments, and emergency response and eradication programs for ALB, LBAM, AGM, emerald ash borer (EAB), *Sirex noctilio* woodwasp, European grapevine moth (EGVM), and other pests. Otis personnel identify high-risk exotic pests and develop survey technology to facilitate the early detection of introductions. The lab continues to support PPQ's gypsy moth 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. Additional work is focused on the development of regulatory treatments for various commodities and means of their conveyance, such as pallets and containers. The Otis Lab is developing rearing

systems for EGVM and ALB and has biological control programs for EAB, *S. noctilio*, and the winter moth.

To fulfill its mission, Otis Lab personnel maintain cooperative relationships with ARS, USDA's Forest Service, universities, and private industry. These cooperative arrangements 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.

Recent Accomplishments

European Grapevine Moth

- Supported the EGVM program by leading a technical working group and formulating 250,000 pheromone lures for the program while commercial sources were being developed.
- Through internal and cooperative projects, developed and/or evaluated phytosanitary treatments that allowed for intrastate, interstate, and international movement of grapes and other EGVM host material; assessed the efficacy of insecticides for EGVM control programs; and confirmed the suitability of attractant-based trapping methods for survey and population monitoring.
- Began developing rearing systems for EGVM for potential use in mass-rearing and determined that the pink bollworm diet was highly suitable for EGVM.

Emerald Ash Borer

- In conjunction with USDA's Forest Service and ARS, successfully released several parasites that attack EAB. Continued to monitor the establishment and effectiveness of these parasites at field release sites and to consult with a PPQ parasite rearing facility in Michigan.
- Collected an additional parasite species in Russia that is under evaluation for field release.
- Continued testing to fine-tune EAB traps and lures, which were developed by the Otis Lab and are used nationally by the EAB program for its survey work. Developed data that will lead to the availability of a dry (non-sticky) trap for EAB within the next 2 years.
- Evaluated EAB dispersal and the systemic insecticide emamectin benzoate in West Virginia.
- Organized the scientific program for an EAB technology research and development meeting and led the effort to organize and edit the resulting compendium of abstracts, which was published by USDA's Forest Service.

Gypsy Moth

- Continued to perform DNA analysis of gypsy moth specimens submitted by PPQ's domestic monitoring program and from specimens intercepted by PPQ and DHS.
- Continued work toward developing a worldwide library for microsatellite DNA markers from gypsy moths, which should prove useful in determining likely source populations for intercepted gypsy moths and newly detected populations. We also began work toward developing DNA "barcoding" capabilities so that intercepted lymantriids that are not gypsy moths can be readily and accurately identified.

Asian Longhorned Beetle

- Continued assessing ALB populations and tree-damage patterns to understand the dynamics and spread of ALB infestations in New York, New Jersey, and Massachusetts. We consulted with the ALB program regarding the age, spread, and survey methodology for the Worcester, MA, infestation and a newly discovered small infestation in Boston.
- Initiated studies to develop regulatory treatments for ALB in nursery stock, as commercial nurseries are now included in ALB-infested areas.

Light Brown Apple Moth

- Supported the LBAM program by leading a technical working group as well as developing and evaluating phytosanitary and control treatments. In particular, we cooperated in finalizing a test of new mating disruption formulations in California, leading to use of the method by caneberry growers in the Monterey Bay area. We also arranged for additional testing of novel mating disruption formulations in New Zealand.
- Continued to develop phytosanitary treatments for LBAM, including fumigation methods for produce and use of systemic insecticides as a means of further reducing the risk of moving LBAM to uninfested areas on nursery stock.

***Sirex* Woodwasp**

- Conducted experimental releases of a biocontrol agent for *Sirex* woodwasp using the nematode *Beddingia siricidicola* in New York and Michigan. Previous field release of

the nematode was evaluated and removed from the field prior to *Sirex* emergence in the summer, but this year we are hoping to continue with an environmental release in order to assess establishment and spread of the nematode under natural conditions.

Survey Support

- Formulated and provided over 50,000 pheromone dispensers (in addition to those for the EGVM program) to support CAPS and other survey efforts. We continued to provide advice on trap, lures, and survey design for these programs.

Phytosanitary Treatments

- Development of new regulatory treatments for pine wood, ash, and firewood is progressing. In particular, good progress was made toward validating the use of radio-frequency waves for phytosanitary treatment of wood, which is nearing acceptance for International Standards for Phytosanitary Measures.

CPHST Lab, Phoenix, AZ

Laboratory Director - Dr. Richard Zink

The Phoenix Lab'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 pink bollworm (PBW), rangeland grasshopper/Mormon cricket complex, and LBAM. These control technologies include biocontrol, the SIT, pheromones, new chemicals, ground and aerial delivery systems, and geographic information system applications. 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 large-scale 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 to maintain state-of-the-art status. The Pink Bollworm section supports the PBW eradication program by providing expertise on pheromone mating disruption, custom rearing and mass-rearing of insects, sterile insect release mechanisms, insect population monitoring, and insect behavior. This section works closely with the PBW rearing facility, the CPHST LBAM Unit and Albany facility, the Arizona Cotton Research and Protection Council, ARS' Arid Lands Agricultural Research Center, the University of Arizona Entomology Department, local cotton growers, the International PBW Eradication Program, and State and regional PPQ offices.

Recent Accomplishments

Rangeland Section

- Conducted research on several domestic strains of the fungal pathogens of *Metarhizium anisopliae* and *Beauveria bassiana* insects in a continuing joint effort with ARS and Utah State University. The *Metarhizium* strains, F52, DWR-356, and DWR-346, as well as the *Beauveria* strain GHA were tested against the Mormon cricket in Sidney, MT, in collaboration with ARS using the Field Aerial Application Spray Simulation Technique on 576 mini-plots. All of the *Metarhizium* strains were tested against grasshoppers on four 10-acre plots near Howell, UT, in collaboration with Utah State University. Both studies are in support of the development and registration of a domestic strain of *Metarhizium* that is suitable for deployment against domestic rangeland pests and were in preparation for future large-scale field trials.
- Applied for a permit to release and evaluate an exotic biopesticide in the western United States for Mormon cricket and grasshopper control. The permit will allow 10-acre applications of Australian Green Guard and African Green Muscle. Tentative plans include application in one of five western States, with the primary location in Montana and secondary sites in nearby States. Techniques for both aerial and ground application have been tested and are available for the trials.
- Conducted an initial evaluation of an aerial application of chlorantraniliprole (Coragen®) to determine a dose range for final evaluation and development for rangeland grasshopper control. This pesticide is a potential alternative to the traditional pesticides malathion and carbaryl.
- Evaluated an adjuvant as a replacement for oil diluents in aerial application mixes of diflubenzuron to control rangeland grasshoppers. If an upcoming operational scale

study is successful and this method is implemented, it will produce significant savings in terms of mixing and loading time, reduced diluent volumes, and aircraft load efficiency.
- Further evaluated and demonstrated the utility of improved low volume and ultra-low volume ground application technology. This evolving technology is being used in several States.
- Evaluated nine new solid bait combinations against the standard carbaryl baits using adult grasshoppers. Coragen®, Bifenthrin, and Indoxacarb will be evaluated on larger scale plots to determine effectiveness against a grasshopper field population.
- Continued efforts to determine the lowest effective dose of Baythroid® XL against field populations of rangeland grasshoppers. The 1-ounce-per-acre rate was as effective as the recommended 2-ounce-per-acre rate, as measured by observed mortality. Material cost and the cost per acre can be reduced without loss of effectiveness. However, high diluent volume is still an economic concern.
- Compared 40-degree flat fan spray tips with standard tips used in rangeland grasshopper/Mormon cricket programs.
- Started a project to develop an inventory for the prevalence of naturally occurring pathogens in rangeland grasshopper populations by surveying untreated field-collected grasshopper cadavers.
- Acquired, catalogued, and made available to researchers thousands of books, technical bulletins, circulars, university-published documents, pamphlets, Web sites, maps, and articles concerning PBW, grasshopper, and Mormon cricket.

Pink Bollworm Section
- Modified a 34-millimeter twin screw extruder to make specialty diets for research and to back up the PBW rearing facility's 80-millimeter extruder.
- Eliminated the preservatives methyl paraben and polysorbate as well as the antibiotic chlortetracycline as potential secondary markers for mass-reared PBW.
- Verified the genetically modified, chlortetracycline-dependent strain OX3402CC as 100 percent unable to reproduce on cotton bolls as designed.
- Completed evaluation on a sprayable formulation of PBW pheromone for mating disruption. The formulation has been applied by a commercial applicator and is ready for product registration.
- Evaluated a genetically modified strain of PBW, OX-1138BB, that performed as well as APHIS rearing facility moths in a comparison of mating competence as indicated by male response to female calling.
- Distributed the PBW bibliographic database to university and ARS scientists to enhance cooperative research efforts.

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 analysis (PRA). 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 both import and export issues and facilitate safe trade. These 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 technical support documents that 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.

Over the years, PERAL has established itself as a global leader in both productivity and quality management. PERAL is currently the only 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 pest risk analysis. Furthermore, PERAL promotes regional and international harmonization of plant health regulations by participating in the North American Plant Protection Organization and the International Plant Protection Convention.

Recent Accomplishments
- Finalized 18 original and 36 revised Q-56 PRA in 2010, facilitating safe trade for 20 commodities from 18 different countries. PERAL also completed 1 original Q-37

PRA and 10 original weed risk assessments and revised 2 PRA that had been completed previously.
- The New Pest Advisory Group (NPAG) analyzed 35 pests and completed an additional 20 preassessments for organisms that were not considered NPAG pests.
- The Exotic Pest Information Collection and Analysis team produced 51 notifications containing 184 articles on pests of regulatory significance.
- Processed risk assessment and informational documents for the organism, pathway, export, and risk mapping areas, including 12 export, 13 organism, 8 pathway, 3 ad-hoc, 1 risk mapping, and 23 special trade support documents.
- The Global Pest and Disease Database team added over 200 new pests to the database.
- Organized and delivered four risk analysis workshops; hosted scientists from Korea as participants in the Risk Analysis Mentoring Program; presented at risk analysis workshops in Ghana, Ethiopia, and Australia; and organized and hosted a pathway symposium for the North American Plant Protection Organization.
- Collaborated with PPQ's Plant Health Programs to develop RegDev, a SharePoint site for the management of regulatory development projects that allows users to request, execute, and track regulatory development work.

Biological Control Unit, Raleigh, NC

Coordinator - Dr. Kenneth Bloem

The CPHST Biological Control Unit (BCU) is a virtual team of 14 to 18 scientists located at various CPHST locations, including Fort Collins, CO; Mission, TX; Otis, MA; Phoenix, AZ; Albany, CA; Moss Landing, CA; Miami, FL; and Guatemala City, Guatemala. The BCU focuses on developing technologies that allow living biological organisms, such as natural enemies and competitors, to mitigate the impacts of introduced invasive insect pests, weeds, and plant pathogens while minimizing impacts on the environment and nontarget organisms.

CPHST scientists provide technical oversight and expertise to programs 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, they 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.

A full listing of the names, project titles, and publications of CPHST scientists working on biocontrol can be found under the highlights for the individual CPHST laboratories, with the exceptions of Amy Roda and Scott Weihman at the Miami Station and Pedro Rendón and Carlos Cáceres at the Guatemala Station, who are administered from the Director's Office in Raleigh, NC.

Recent Accomplishments

Asian Citrus Psyllid

CPHST Mission developed protocols for mass-production of *Tamarixia radiata*, a parasitoid of ACP, using a field insectary cage approach. In addition, new strains/collections of *T. radiata* from Pakistan, Guangxi, Guangdong, Guangzhou, and Hong Kong were established in quarantine, and host range testing was initiated.

Harrisia Cactus Mealybug

The Harrisia cactus mealybug is a serious pest of columnar cacti. It is established in Florida, but it appears to be under good biological control. In Puerto Rico, it is causing significant damage to the endemic and endangered columnar cacti of the Guánica Dry Forest Reserve. CPHST Mission and Miami conducted surveys in Florida, Puerto Rico, and Barbados and found a parasitoid wasp tentatively identified as *Leptomastidea* nr *antillicola* Dozier (Hymenoptera: Encyrtidae) attacking the pest, which may serve as a classical biological control agent.

Red Palm Weevil

CPHST Miami determined distribution, population densities, survey methodologies, control options, and impact of the red palm weevil, a recently introduced pest of palms in Aruba and Curaçao. Results of these studies were used to develop the New Pest Response Guidelines for this pest. These recommendations are now being used to combat the red palm weevil infestation recently detected in southern California.

Emerald Ash Borer

CPHST Otis continued to evaluate the establishment, spread, and impact of exotic parasitoids being released for EAB control in cooperation with scientists from USDA's Forest Ser-

vice and ARS. The three species of *Oobius*, *Spathius*, and *Tetrastichus* were found to have successfully overwintered at sites in Michigan, Ohio, and Maryland. CPHST Otis also discovered and initiated studies of new natural enemies from South Korea and the Russian Far East.

Mediterranean Fruit Fly
CPHST Guatemala continued to conduct field tests to evaluate and optimize combined releases of the egg parasitoid *Fopius ceratitivorous* and sterile Medflies. Ground releases of the parasitoid alone suggested that Medfly populations could be reduced by 40 to 60 percent. Results from combined releases are still being analyzed in an effort to establish a recommended release density of the parasitoid.

Light Brown Apple Moth
The LBAM Unit is collaborating with the California Department of Food and Agriculture to assess the ability of the egg parasitoid *Trichogramma platneri* to suppress LBAM populations alone and in combination with sterile moth releases. Field cage tests suggested that both techniques have potential for LBAM control.

Grasshoppers and Mormon Crickets
CPHST Fort Collins and Phoenix, in a continuing joint effort with ARS and Utah State University, initiated field studies on several domestic strains of fungal pathogens, *Metarhizium anisopliae* and *Beauveria bassiana*, for control of grasshoppers and Mormon crickets. In addition, CPHST Phoenix applied for permits to release and evaluate the exotic biopesticides Green Guard® from Australia and Green Muscle® from Africa, which are based on different strains of *M. anisopliae*.

Imported Fire Ant
CPHST Gulfport continued to coordinate the APHIS-funded IFA Phorid Fly (*Pseudacteon* spp.) rearing and release program. In 2010, multiple releases of a third fly species, *P. obtusus*, and production rearing of a fourth species, *P. cultellatus*, were initiated. The first 2 species, *P. tricuspis* and *P. curvatus*, are now established in more than 50 percent of the IFA quarantined area.

Knapweeds
CPHST Fort Collins and the Albany work unit continued their efforts to optimize a rearing system for *Cyphocleonus achates*, a root-feeding weevil used for biological control of knapweeds. Studies indicated that stockpiling adults in reproductive diapauses may be possible for up to 3 months by manipulating light cycles.

Russian Knapweed
A colony of the new Russian knapweed biological control agent, the gall midge *Jaapiella ivannikovi*, was initiated at the Fort Collins Laboratory in 2010. Overwintering and establishment of the midge at 2009 release sites in Wyoming and Montana were confirmed, and new releases were made at several sites in Colorado.

Perennial Pepperweed and Canada Thistle
CPHST Fort Collins initiated host specificity testing of a new race of white rust, *Albugo candida*, that is attacking perennial pepperweed from collections made in Colorado and California. CPHST Fort Collins also facilitated a survey for fungal pathogens of Canada thistle in northwestern China through a cooperative agreement with CABI Europe-Switzerland.

Fruit Fly Unit, Raleigh, NC

Coordinator - John Stewart

The Fruit Fly Unit supports the APHIS Fruit Fly Exclusion and Detection program's major goal to strengthen detection and response capabilities, preventative release programs, and control programs in order to prevent exotic fruit fly populations from becoming established and/or spreading within the United States. The Fruit Fly Unit uses local program and international review recommendations to guide the development of methods support activities. Currently, the unit is working on recommendations from expert reviews on APHIS fruit fly detection programs, fruit fly eclosion and release facilities, and the Lower Rio Grande Valley Eradication project. CPHST has increased fruit fly program support by adding positions for a Fruit Fly Unit coordinator, as well as entomologists in the Eclosion and Release Centers in Sarasota, FL, and Los Alamitos, CA. The unit has developed workplans that will address the science portions of the review recommendations as well as meet the local needs of the program experts in the areas of fruit fly detection, control, and preventative releases against the major exotic fruit flies of concern.

Recent Accomplishments

- Developed the methods support to evaluate solid formulations of methyl eugenol and cue lure for purchasing contract purposes, which could garner large program

savings and address safety concerns.

- Determined the efficacy of three- and two-component female trap lures for contract purposes and for direct comparison against Medfly and *Anastrepha* species.
- Determined that the use of the three-component lure is valid for Florida's detection program that targets Medfly and also for *Anastrepha* species as a secondary concern.
- Determined that the Mexfly program in south Texas and northern Mexico could gain efficiency by adopting the two-component solid formulation for contract purchases.
- Led the National Trapping Committee in the review and development of new traps and lures and the coordination of testing for new lures and new formulations of lures.
- Validated the program's use of ginger root oil as a tool for improved sterile male Medfly performance in sterile release facilities.
- Developed a prototype novel bait station that is effective, persistent, and inexpensive and can be used in environmentally sensitive areas with good results.
- Developed the APHIS/Cooperator SIT Rearing and Eclosion Quality Assurance Web site for fruit fly rearing and release data compilation and analysis from all U.S.-supported SIT facilities.
- Initiated and chaired the Rearing and Eclosion Working Group in order to analyze quality assurance data and to address review recommendations and quality control issues within this key program component.
- Developed a Black Pupae Strain for Mexfly release programs that could eliminate the release of females and provide increased efficiency within the Mexfly eradication program.
- Conducted sterile male competitive testing with wild fruit flies in Guatemala and Texas (under quarantine) for Medfly and Mexfly to ensure program effectiveness.
- Developed microbial identification and decontamination procedures in the Mexican Fruit Fly Rearing Facility in Mission, TX that vastly improved the facility's mass-rearing of these insects.
- Initiated the *Anastrepha* Molecular ID Project for passenger baggage for improved pathway analysis.
- Validated different rates of Spinosad GF-120 in the Moscamed Gradual Advance Plan program, which led to the program adoption of lower application rates and resulted in large cost savings.
- Tested and validated the use of a new double-box release machine for the Lower Rio Grande Valley Mexfly Eradication project, which will result in significant program savings and flight time.
- Developed methodology to evaluate sterile to wild overflooding ratios in the Guatemala Moscamed program, which allowed more efficient utilization of the sterile Medfly resources within the Moscamed Barrier program.

Light Brown Apple Moth Unit, Moss Landing, CA

Coordinator - Dr. Gregory Simmons

The LBAM Unit supports the CPHST mission by coordinating and conducting scientific and technical support activities in response to the 2007 detection of LBAM in California. The unit also supports USDA and the California Department of Food and Agriculture's (CDFA) mission of controlling and managing LBAM to prevent its spread. The main focus of the unit is to develop the technology needed to implement the SIT for LBAM suppression. This work includes methods development and the evaluation of technology for mass-rearing, irradiation, handling, release, and monitoring of sterile LBAM. Field and laboratory evaluations are conducted to measure the mating competitiveness and field performance of released insects, which are the major factors that determine the effectiveness of the SIT. A coordination of efforts among the LBAM Unit and Fort Collins, Mission, Otis, and Phoenix laboratories has resulted in advancements in identification, detection, and control of LBAM. Experiments are also being performed to better understand LBAM's ability to spread and disperse to new areas. The unit also supports USDA and CDFA's efforts to detect, delimitate, and control the EGVM (*Lobesia botrana*) in California.

Recent Accomplishments

- Made several advancements in LBAM mass-rearing that increased system production and efficiency, allowing stable levels of production to exceed 100,000 moths per week and increased egg production and quality. Factors affecting the growth of pathogens in the rearing system were identified for improved sanitation. These efforts have set the stage for expanding the weekly production in support of field evaluation trials in 2011.
- Added an additional 1,600 square feet of rearing space and refined the adult collection system, allowing increased collection efficiency and production capacity.
- Validated cold treatment for the control of LBAM for movement of strawberry plants for planting.
- Worked with ARS and the Otis Lab to test methyl bromide and alternative fumigants for LBAM post-harvest

control for several commodities, including apples, stone fruits, and caneberries.

- Conducted studies with CDFA to test the combined effects of sterile insect release with the egg parasitoid *Trichogramma platneri* on suppression of LBAM in field cages. These results showed that LBAM could be reduced by as much as 98 percent by a single release of sterile moths with parasitoids in field cages. Cooperative work with CDFA is planned for 2011 as part of the SIT field evaluation project.
- Supported the EGVM control program by conducting studies of phenology and surveys of alternate host plants in Napa County; conducting a demonstration of mating disruption for control of EGVM on a small isolated population; testing monitoring methods for areas under areawide control using mating disruption; and testing different delta traps and pheromone lure formulations for specification of contract purchases for the program.
- Conducted research using LBAM as a model organism for EGVM to assess the effects of winemaking procedures on the survivability of larvae and pupae during crushing, pressing, and storage of grape must for shipping. Over 2,000 pounds of wine grapes were processed, and several thousand larvae and pupae were tested. This work has allowed refinements to the current regulations regarding the treatment of winemaking green waste, pressing procedures, and the shipment of red wine must.

Treatment Quality Assurance Unit, Raleigh, NC

Director - Scott Wood

The Treatment Quality Assurance Unit (TQAU) develops, adapts, and supports technology to detect, identify, and mitigate the risk posed by exotic pests in preclearance programs and at ports-of-entry. APHIS' last line of defense in preventing an exotic pest invasion is the commodity treatment that mitigates this risk. Currently, TQAU's core activities include developing quarantine treatments, contributing to the treatment manual for ports-of-entry, certifying shipping containers and vessels, maintaining the Commodity Treatment Information System (CTIS), training-the-trainer for proper application of treatments, certifying commodity treatment facilities within foreign countries, and conducting quality assurance audits of treatments performed domestically and at foreign sites.

Recent Accomplishments

- Developed two new databases for the CTIS: a new version of the Fumigation Form 429 Database and an export module for the Irradiation Reporting and Accountability Database.
- Continued support and expansion of remaining CTIS sites for APHIS and industry use, including the 556 In-Transit Cold Treatment Database, Niger Seed Database, the Irradiation Reporting and Accountability Database, the Treatment Index Database, and the Vessels and Containers Database.
- Documented port methyl bromide fumigation use patterns and commodity movement post-aeration for asparagus and cut flowers in Miami, FL.
- Compared infrared and thermal conductivity methyl bromide concentration monitors and provided operational guidance regarding purchasing and use.
- Recertified irradiation facilities in India; approved mango hot water facilities in Mexico, Brazil, Peru, Guatemala, and Haiti; approved fumigation chamber in the United States and South Korea; approved cold treatment warehouses in South Korea; approved and certified a Niger seed facility in Ethiopia; and recertified Niger seed facilities in the United States.
- For in-transit cold treatment of fruit: certified 113,239 self-refrigerated containers; certified 50 vessels; approved plans for 4 new vessels; approved 8 warehouses; approved 20 new types of equipment; and approved installation of new equipment on 5 vessels.
- Maintained existing ISO 9001:2008 registration for cold treatment and irradiation processes and added a new irradiation process to the ISO registry. Developed an application for fumigation chambers to be used in future ISO treatment.
- Continued electronic indexing of documents in the TQAU library in an effort to develop a paperless electronic system for AQI treatments, policies, and research literature. The multiple-year process has accumulated a grand total of 17,000 documents.
- Developed new heat treatment of dried plant material for plant pathogen bacteria.
- Developed new hot water treatment for propagative plant material not tolerant to fumigation.
- Developed new cold treatment for snails on food/feed commodities.
- Worked with the PPQ Professional Development Center to create training materials for cold treatment vessels

inspection, mango hot water programs, irradiation programs, and the Fumigation Form 429 Database.
- Provided over 150 treatment recommendations for PPQ officers and stakeholders. The historical references and all new recommendations are now held in the newly-developed Endnote Treatment Recommendation Library.
- Approved control atmosphere bags for cold treatments of blueberries.
- Approved a new process for conducting containerized log export fumigations.
- Reviewed quarantine treatment research for high-risk pests, including Queensland fruit fly (*Bactrocera tryoni*), Medfly (*Ceratitis capitata*), mango pulp weevil (*Sternochetus frigidus*), false codling moth (*Thaumatotibia leucotreta*), Natal fruit fly (*Ceratitis rosa*), spotted wing drosophila (*Drosophila suzukii*), ACP (*Diaphorina citri*), EAB (*Agrilus planipennis*), Asian fruit fly (*Bactrocera invadens*), EGVM (*Lobesia botrana*), Chilean false red mite (*Brevipalpus chilensis*), LBAM (*Epiphyas postvittana*), oak wilt (*Ceratocystis fagacearum*), and sweet potato weevil (*Cylas formicarius*).
- Developed research protocols aimed at establishing methyl bromide, cold, hot water, vapor heat, and ozone treatment schedules for high-risk pests, including Chilean false red mite, EGVM, false codling moth, litchi moth (*C. peltastica*), Medfly, South American fruit fly (*Anastrepha fraterculus*), West Indian fruit fly (*A. obliqua*), guava fruit fly (*A. striata*), coffee berry borer (*Hypothenemus hampei*), and coffee leaf rust (*Hemileia vastatrix*).
- Participated in the Treatment Recommendation and Reconditioning Agricultural Risk Management Projects. All TQAU staff members received a certification of appreciation from PPQ's Plant Health Programs for participation.
- Reviewed treatment proposals for the International Plant Protection Convention Technical Panel on Phytosanitary Treatments.
- Provided support to the Quadrilateral Scientific Collaboration Work Group and project teams (United States, Canada, Australia, and New Zealand).
- Developed and approved process designs for containerized and large-scale log and produce fumigations.
- Evaluated new technologies for remote monitoring (monitoring from extended distances outside of a buffer zone) for chemical and non-chemical treatments and aeration.
- Evaluated the effects of irradiation on blueberries and peaches for export to Mexico, which was funded by a Technical Assistance for Specialty Crops grant from USDA's Foreign Agricultural Service.
- Compiled extensive methyl bromide usage studies for 2005 through 2010, including data on the commodity, country of origin, reporting station, schedule, and contractor.

National Plant Pathogen Laboratory Accreditation Program, Raleigh, NC

Coordinator - Dr. Patrick Shiel

The National Plant Protection Laboratory Accreditation Program (NPPLAP) evaluates laboratories conducting molecular diagnostics for PPQ to ensure their capability to make accurate diagnostic determinations for regulatory purposes. In addition to ensuring laboratory capability within PPQ and other USDA agencies, NPPLAP engages the National Plant Diagnostic Network (NPDN) and State agricultural department laboratories in this process to increase diagnostic capacity and proficiency in a dispersed laboratory network. This program supports the PPQ mission by establishing a state-of-readiness when needed for emergency situations. NPPLAP currently accredits laboratories to diagnose *Phytophthora ramorum* and the HLB (citrus greening) pathogen. NPPLAP is also involved in the advanced diagnostics used for the regulatory response to the SOS pathogen (*Elsinoë autralis*) and the CBS pathogen (*Guignardia citrocarpa*) introductions. NPPLAP is currently developing a program suitable for USDA regulatory testing of *Plum pox virus* for the current eradication program as well as national surveys.

NPPLAP promotes accreditation standards suitable for use by plant diagnostic laboratories and development of a Quality Management System for plant diagnostic labs. Recent engagements with the American Association of Veterinary Laboratory Diagnosticians (AAVLD) accreditation program and the NPDN have been fruitful in accelerating incorporation of Quality Management principles for plant diagnostic testing. One of the main objectives for NPPLAP is the continued engagement and training in Quality Management principles to support a functional accreditation system for plant diagnostics.

Recent Accomplishments

- Facilitated alignment of the NPDN Quality Management System with the established AAVLD system. Developed a template Quality Manual for use by NPDN laboratories and hosted the NPDN for training and document development.

- Completed the *P. ramorum* Proficiency Test program for the fifth year.
- Completed the HLB Proficiency Test program for the third year.
- Deployed a *Plum Pox Virus* Beta Proficiency Test for NPPLAP implementation.
- Added five new participating laboratories in the NPPLAP system for 2010.
- Developed reaccreditation criteria and processes for participating laboratories.

Project Highlights

Citrus leprosis virus Molecular Detection and Identification

Location: CPHST Beltsville Laboratory
Lead Scientist: Mark Nakhla and Wenbin Li
Team Members: Gang Wei
Cooperators: Ron Brlansky, University of Florida

Citrus leprosis virus (CiLV) was originally detected and described in Florida over 100 years ago and was later found in Paraguay, Uruguay, Argentina, and Brazil, where it is endemic. Recently, the disease was detected in Bolivia, Venezuela, Colombia, Panama, Costa Rica, Nicaragua, Guatemala, Honduras, El Salvador, and Mexico. The disease has not been observed in the United States since the 1960s, but its presence in nearby countries presents a risk for reintroduction. CiLV produces chlorotic lesions with or without necrotic centers on citrus leaves, fruit, and twigs and can cause serious crop losses, with sweet orange and mandarins being the most severely affected citrus types. The virus is transmitted by *Brevipalpus* spp. mites, and two types of CiLV have been described. The virus particles of the most prevalent "cytoplasmic type" are found mainly in the cytoplasm, while virus particles of the other "nuclear type" primarily reside in the nucleus of infected cells. Only the CiLV-cytoplasmic type (CiLV-C) has been molecularly characterized, and it is classified in the novel *Cilevirus* virus genus.

We developed two multiplex one-step conventional polymerase chain reaction (PCR) protocols for the detection and identification of CiLV-C in citrus plant samples. One of these protocols is based on the CiLV-C-specific published primers MPF and MPR, which amplify a 339-base pair fragment from the movement protein gene.

As a rapid screening tool for surveys, we developed a multiplex one-step real-time PCR protocol for the detection and identification of CiLV-C in citrus plant samples. This real-time PCR targets a segment of the movement protein gene using CiLV-specific primers and a fluorescently labeled probe. The laboratory designed the reverse primer and the probe, while the forward primer was modified by the laboratory based on the published conventional primer MPF. We combined these virus-specific primers and probe with primers and a probe for the Nad 5 plant gene as an internal control. Using this multiplex one-step real-time PCR assay, we successfully detected CiLV-C in citrus samples from Costa Rica and Panama.

This project made substantial progress in 2010, and we are currently validating the assay using field materials from Central America. We will begin work to detect the virus within its mite vector in the next year. We will also be providing the protocol to international partners and offering two hands-on training courses in the spring of 2011 for State and Federal diagnosticians.

Development of Waste Disposal and Decontamination Methods for International Trade and Emergency Agricultural Needs

Location: CPHST Fort Collins Laboratory
Lead Scientist: Craig Ramsey

The United States imports more than $40 billion worth of agricultural commodities annually, and more than 430,000 people travel to the United States each day. There are 327 federally regulated ports and border stations, with 15 preclearance sites for agricultural imports into the United States. Disposal of seized agricultural products requires sanitation methods that are inexpensive, flexible, safe for the environment, and highly effective against all types of pests and diseases. The cost of regulated airline waste incineration at international airports was about $2,600 per ton in 2009 when handled by a waste disposal vendor. The CPHST Fort Collins Lab conducted studies on two alternative waste disposal and chemical decontamination methods in 2010.

A preliminary decontamination test with a chlorine dioxide disinfectant (Electro-biocide) was conducted by a private microbiology laboratory. The results show 100-percent deactivation within 10 minutes at 200-parts-per-million chlorine dioxide or a 6.5 log reduction in colony-forming units (CFU) for *Bacillus subtilis*, a spore-forming bacterium. Future disinfectant testing will include hydrogen peroxide formulations and on-demand generators for chlorine dioxide and ozonated water, which can be formulated as oxidant/adjuvant mixtures (gas dissolved in water) onsite. Commercial technology also exists to use these oxidants as gas phase fumigants. Sprayers are available to apply these formulations as compressed air foam, thermo-fogging, or electrostatic applications.

The Fort Collins Lab also tested the use of alternative, more cost-effective waste disposal machines that are currently used by hospitals and research labs. The study objectives included estimating operational costs and determining the seed devital-

ization and microbial efficacy for both machines. BioSAFE Engineering manufactures a shredder/steamer that shreds waste into 7-millimeter particles, which is then steam-treated in a jacketed auger at 212 ºF and 13 pounds per square inch (PSI) for 30 to 40 minutes. BioSAFE also manufactures an alkaline chemical digester, which operates at 300 ºF and 60 PSI and uses 18-percent potassium hydroxide as the reductive agent. The chemical digester converts biological tissue, except for cellulose-type materials, into a liquid similar in appearance to vegetable oil.

Both the steam and digester tests involved a microbial efficacy test, which was analyzed by a private laboratory, and a plant seed devitalization test. These tests resulted in complete devitalization of 10 species of plant seeds. The microbial efficacy test showed that following approximately 30 minutes of steam treatment, there was a six-log CFU reduction for about 60 to 80 pounds of hay. The results for the digester showed complete deactivation of all microbes for 60 liquid samples. The digester reduced the 245 pounds of research animal waste used in this study to a sterile, non-infectious, hydrocarbon liquid.

Three waste disposal and chemical decontamination projects are planned for 2011. The waste disposal study will include retesting the steamer and chemical digesters with *Bacillus subtilis* added to a mixture of plant and animal waste to mimic real-world conditions. Optional tests may include a shredder and lime technology currently being considered by U.S. Environmental Protection Agency (EPA) officials. Decontamination testing will be continued to test oxidants with foam and electrostatic sprayers. In addition, microbial efficacy testing will be extended to include a variety of surfaces, with and without organic challenges, and selected plant pathogens, nematodes, and insect eggs.

Development of Cost-Effective Extraction Method for Analysis of Diflubenzuron in Vegetation

Location: CPHST Gulfport Laboratory
Lead Scientist: Lisa Mosser
Team Members: Gulfport Lab Chemistry Staff
Cooperators: Robert Baca, PPQ Emergency and Domestic Programs

In March 2010, work was completed on the development and validation of a cost-effective and "green" wet chemistry extraction for the determination of diflubenzuron in vegetation. The APHIS grasshopper program requested a method to analyze vegetation samples. Prior methods available for determining diflubezuron in vegetation did not effectively address matrix interferences, resulting in poor recovery data. Therefore, development of a replacement method was needed immediately to address program needs. In general, the grasshopper program uses the data generated by the Gulfport Lab on submitted samples as a means of fulfilling its commitment to monitoring environmental stewardship of its chemical treatment programs. Specifically, vegetation samples submitted by the grasshopper program often represent sensitive claims/issues related to "spills" during pesticide application in the field or "drift events" into organic food growing or residential areas.

CPHST needed a new platform to address the grasshopper program's specific need for a method that processed vegetation samples and produced analytical results with sustained confidence. Steve Lehotay of USDA's Agricultural Research Service had developed a chemical assay for the "Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Approach for Determining Pesticide Residues" in food matrices. The laboratory decided to adapt this "food" extraction technique for applications using vegetation. Vegetation is a challenging matrix as it often contains little moisture and is highly variable, as compared to foods that are consistent and often contain high moisture content.

A successful extraction method using a modified QuEChERS technique was developed in-house by the Gulfport Lead Chemist. This was coupled with a new liquid chromotography/mass spectrometry (LC/MS)-based detection scheme applied to the extract for determining and confirming diflubenzuron in vegetation. The method was then validated to ensure consistent application to program samples. The modified extraction developed as part of the overall method requires only 12 milliliters of organic solvent and produces about 12 milliliters of associated type "A" hazardous waste per sample. The previous extraction method required approximately 240 milliliters of solvent and generated around 240 milliliters of type "C" hazardous waste per sample. Thus, we were able to reduce overall waste solvent by 95 percent and introduce less expensive Type "A" waste in our waste management system. This translates into cost savings due to reduced solvent use and lower hazardous waste disposal costs, as well as a lower environmental impact due to a reduction in hazardous waste generated. This extraction method is also less hazardous for

the wet chemistry staff to handle during sample processing, and the extraction technique is easier and faster to conduct. Utilization of highly selective LC/MS detection allowed us to produce confirmed data results in a dirty matrix that historically contained interferences mimicking the compound of interest. Due to the success of this method, we plan to use the modified QuEChERS extraction developed for the grasshopper vegetation samples on vegetation samples submitted by other programs. We will also expand application of this technique to include other matrices such as water and soil.

Molecular Tools for Identification of Light Brown Apple Moth

Location: CPHST Mission Laboratory
Lead Scientist: Norman B. Barr
Team Members: Lisa A. Ledezma, Roxanne E. Farris
Cooperators: Todd Gilligan (Colorado State University), Marc Epstein (California Department of Food and Agriculture), Richard Newcomb (New Zealand Institute for Plant and Food Research)

The light brown apple moth (LBAM), *Epiphyas postvittana*, is an invasive pest of apples, citrus, and grapes in Australia and New Zealand. It also has the potential to damage many additional agricultural and horticultural commodities. To date, LBAM has been recorded feeding on more than 500 plant species belonging to 121 families.

Native to Australia, LBAM has successfully established in New Zealand, Hawaii, the British Isles, and most recently California. Although reported from the Hawaiian Islands since the 1890s, LBAM was confirmed in California for the first time in 2007.

The presence of LBAM in California has raised several important questions from Federal and State regulatory agencies, including:

- What is the origin of the LBAM population in California?
- How did the population enter California?
- How is the moth population moving within the State?
- How can we identify moth eggs and caterpillars detected within California when traditional tools of identification require adult moths?

Using DNA analyses, we are able to develop new ways of addressing these questions.

In 2010, the California Department of Food and Agriculture, the New Zealand Institute for Plant and Food Research, Colorado State University, and the CPHST Mission Lab collaborated on projects to develop DNA tools for LBAM molecular identification and pathway analysis. Our DNA analysis of worldwide LBAM populations shows that the population in California did not originate from either the Hawaiian or British populations. Australia and New Zealand are still considered possible sources of the population in California.

In addition, we developed a real-time PCR assay for identifying LBAM samples by DNA analysis. The assay has been tested on both worldwide LBAM collections, demonstrating its reliability, and additional *Epiphyas* species from Australia, demonstrating its specificity. The technique has been released as a work instruction for agency use.

In comparison to other DNA tools developed for LBAM identification, the real-time PCR assay is fast and inexpensive. For example, it takes at least 48 hours to diagnose an LBAM sample using a technique called DNA barcoding. A conventional PCR assay, developed at our lab to reduce assay time, requires 3 to 4 hours to diagnose a DNA sample. The new real-time PCR assay can detect LBAM DNA in less than an hour, which represents a significant reduction in processing time for laboratories and cost savings for the agency.

Our newly developed molecular tools can identify moths collected in nurseries, in commercial groves, or during pest surveys designed to document its distribution. The methodology can also be used to detect novel DNA types within California that could suggest new introductions. Further investment in DNA techniques will assist in tracking the movement of LBAM populations within the United States.

Development of Phytosanitary Treatments for Invasive Tortricids in California

Location: CPHST Otis Laboratory
Lead Scientist: Scott Myers
Team Members: Hannah Nadel, Sian Bailey, Tonya Dockray (Otis Lab); Peter Taverner (Entomologist, SARDI, Adelaide, Australia); Spencer Walse (USDA-ARS, Parlier, CA); Yong-Biao Liu (USDA-ARS, Salinas, CA); Greg Simmons (CPHST LBAM Unit)

The recent establishment of two exotic tortricid moths, LBAM (*Epiphyas postvittana*) and European grapevine moth

(EGVM) (*Lobesia botrana*), has necessitated the development of new phytosanitary treatments for a number of important agricultural industries within the United States.

LBAM, which was first confirmed in California in 2007, is highly polyphagous and is known to damage a number of economically important crops, making it a significant threat to U.S. agriculture. Current regulations require insecticide treatments when detections are made at a nursery for all plants prior to shipment outside of the quarantine area. The goal of this project has been to develop treatments for the many industries affected by these two insects. For LBAM, our focus has been to (1) identify reduced risk insecticide treatments for eggs and larvae that are efficacious and safe to workers and consumers and (2) develop methyl bromide fumigation schedules that will allow for continued export of commodities from the fruit production areas in California impacted by this pest. Working with a variety of State, Federal, and international cooperators, we have conducted a number of field and laboratory experiments to establish treatments for nurseries, fruit, and berry producers as well as quarantine import and export requirements.

EGVM is a severe pest of grapes that was first confirmed from fall 2009 collections in Napa, CA. Many growers experienced damage to their crops, and populations were high enough in some vineyards to prevent the grapes from being harvested for wine production. As a result, State and Federal regulations prohibit the shipment of grapes and other plant parts from areas where EGVM is known to be present unless an effective treatment is applied. Working with a laboratory culture of field-collected pupae, our research has focused on developing effective methyl bromide fumigation treatments for quarantine use.

Accomplishments for LBAM

- To date, CPHST has made treatment recommendations to the LBAM program in California, which include conventional and organic insecticide formulations of *Bacillus thuringiensis*, spinosad, and horticultural oils. In addition, we have recommended the addition of the insect growth-regulator methoxyfenozide for larval control and the use of this chemical in combination with spray oil for control of egg and larval stages. Together, these products have given the nursery industry more choices for regulatory use that are safer for workers and less harmful to beneficial insects.
- Collaborating with USDA's Agricultural Research Service (ARS), we have developed efficacious methyl bromide treatment schedules for grapes, stone fruit, caneberries, and apples that will allow the movement of U.S. produce from LBAM quarantine areas.
- Ongoing work on this project is focusing on evaluating products with systemic activity against early instar larvae. These treatments would provide an additional level of security for plants shipped from quarantine areas.

Accomplishments for EGVM

- Developed methods for the large-scale production of EGVM to conduct phytosanitary treatment research.
- Provided recommendations for hot water, composting, deep burial, and processing treatments for grapes, pomace, and other waste products for movement outside quarantine areas.
- Developed treatment schedules for grapes (T101-h-2) to allow chamber fumigations with methyl bromide as well as tarpaulin fumigations with methyl bromide (in conjunction with cold storage). These treatments have allowed uninterrupted shipment of grapes from Chile during winter months in the United States.
- Continued to conduct work to expand fumigation schedules in order to provide effective treatments at a range of temperatures. These treatments will provide the domestic table and wine grape industries with more flexibility when choosing treatment options. In addition, we plan to explore methyl bromide alternatives in FY 2011.

Longevity of a Fluorescent Visible Marker and a Genetic Marker in Genetically Engineered Pink Bollworm

Location: CPHST Phoenix Laboratory
Lead Scientist: Michelle Walters
Team Members: Guolei Tang, John Claus, Tom Kalaris, Neil Morrison, Caroline Phillips

Pink bollworm (PBW) is an invasive exotic pest capable of causing crop failure in cotton. In order to eradicate this pest, the PBW eradication program employs the sterile insect technique (SIT), in which millions of irradiated, sterile PBW moths are released to mate with the wild population and reduce the production of viable offspring. The program places sticky Delta traps near each cotton field to monitor populations for wild and mass-reared moths. The program must be able to differentiate a wild moth from a mass-reared, sterilized

moth to determine if the eradication effort is succeeding. For this reason, the PBW rearing facility includes Calco red dye in the insects' artificial diet. The dye remains in the adult insect's abdomen and is used as a visual indicator of the origin of the trapped insect. However, it is not 100-percent persistent and is not inherited by offspring. Oxitec LTD (Oxford, England) and CPHST Phoenix developed a genetically modified PBW, designated as OX1138BB, that carries a transgene that expresses red fluorescent protein. OX1138BB was evaluated in large-scale rearing and field trials. This red protein can be viewed in the moths when exposed to a specific type of light, and a goal of the evaluation was to determine the length of time that the fluorescent marker remained visible in moths in a sticky trap in the field.

For this test, all moths were reared on a standard PBW diet that included the Calco red dye. Male moths were irradiated and were placed individually in one of 60 grids in a marked trap. For each trap, between three and seven moths were wild-type (mass-reared) moths and the remainder were OX1138BB. The position of each moth was recorded on a key. Each trap was marked with a unique number on the outside and placed in the field cage in a cotton crop. One person prepared the traps, and a second person screened the traps.

All of the traps were screened and data was recorded at 3-day intervals. In each replicate, all moths retained fluorescence for 1 week and 89 to 99 percent retained fluorescence at 2 weeks. Traps were held in the field for up to 34 days or until visual fluorescence dropped to below 40-percent accuracy. The primary factor affecting correct visual identification appears to be temperature.

At the conclusion of the field study, 300 moths were analyzed for the transgene insertion using PCR. The technician conducting the analysis had no prior knowledge of individual moth strain. PCR analysis was not affected by temperature nor exposure duration. In our blind study, PCR genotyping of 300 samples was 100-percent correct in identifying APHIS wild-type versus OX1138BB moths.

The genetic modification that led to a fluorescent visual marker, backed up by PCR analysis, appears to be a very reliable means of separating wild-type from genetically marked moths. In the eradication program, traps are left in the field for 1 week. Even under very hot conditions, the genetically marked fluorescent insects are expected to be almost 100-percent visually identifiable. If the OX1138BB were mass-reared and released, they would also be fed Calco red dye, which is estimated to be at least 95-percent reliable for identifying mass-reared moths. The two markers are independent, so if each were only 95-percent reliable, then the probability of at least one marker remaining visible is 0.9995 percent. If neither marker were visible, PCR analysis is another method that provides highly accurate identification, even after many weeks in the heat.

Evaluating Plant Health Risks Associated With a Proposed U.S. Military Buildup on Guam

Location: CPHST Plant Epidemiology and Risk Analysis Laboratory
Lead Scientist: Heike Meissner
Team members: Robert Ahern, Thomas Culliney, Andrea Lemay, Ashley Hiser, Lisa Kohl, Olivia Lenahan, Alonso Suazo, Yu Takeuchi

The U.S. Department of Defense (DoD) is planning an extensive military buildup on Guam. This buildup will lead to a significant increase in Guam's population, together with a rise in traffic, trade, and construction activities. Guam and the rest of the Micronesian Region will be affected.

Guam has undergone extensive development, particularly over the last 60 years. Numerous exotic species have been introduced over time and have taken their toll on Guam's native ecosystems. The rest of the Micronesian Region, however, possesses a more intact natural environment with numerous indigenous plant and animal species existing on many of the islands. Guam serves as a gateway to the Micronesian Region, and any exotic species introduced into Guam may reach other parts of the region.

Although agriculture is not a particularly large economic sector in Guam or the rest of the Micronesian Region, subsistence farmers rely on crop production, and some plant species have great cultural value. Most notable among these agriculturally and culturally important plants are coconut, numerous varieties of banana, taro, papaya, avocado, breadfruit, and mango. Therefore, the introduction of pests affecting any of these plant species may have a serious impact on the region's people and culture.

The DoD requested that APHIS, the U.S. Geological Survey (USGS), and the Smithsonian Institution assess the likelihood and consequences of exotic species introductions due to the

proposed military buildup and to develop biosecurity plans to mitigate the identified risks. The National Invasive Species Council was responsible for overall coordination of this effort. The scope of the assignment included the Micronesian Region and Hawaii, with focus areas being terrestrial pests (APHIS), freshwater species (USGS), and marine organisms (Smithsonian).

The APHIS risk analysis was conducted collaboratively by CPHST, Wildlife Services, and Veterinary Services, while PPQ Quarantine Policy Analysis and Support was charged with the development of the APHIS biosecurity plan. With a budget of over $1.2 million and some 25 core team members, the risk analysis likely constitutes the largest such collaboration carried out by APHIS thus far.

The result of this collaboration was a 600-page risk analysis, which was completed on time and on budget. CPHST played a key leadership role in this undertaking, providing considerable expertise in planning, communication, and project and document management.

The APHIS risk analysis is organized into four main sections discussing the risk to plant pests, terrestrial vertebrates, wildlife diseases, and livestock diseases, respectively. The plant pest section follows a qualitative approach, discussing risk for the following pathways of introduction: movement of people; containers, conveyances, and equipment; wood-packaging material; construction materials; plant propagative material; mail; agricultural commodity imports; and garbage. In addition to an assessment of risk, the document provides a list of suggested safeguarding measures as input for the APHIS biosecurity plan.

Biological Control of Tropical Soda Apple in Florida, Georgia, Alabama, and Texas Using the Leaf-Feeding Beetle *Gratiana boliviana*

Location: CPHST Biological Control Unit
Lead Scientist: Dr. Amy Roda
Team Members: Scott Weihman (Miami BCU), Dan Flores (Mission), Daniel Martinez (Mission), Jose Renteria (Mission), Eustorjio Rivas (Mission)
Cooperators: University of Florida (Gainesville, Ft. Pierce, Homestead), PPQ Florida, PPQ Alabama, PPQ Georgia, PPQ Texas, Georgia Department of Agriculture, USDA-ARS (Tallahassee), Florida Division of Plant Industry, Florida A&M University

Tropical soda apple (TSA), *Solanum viarum*, is an invasive perennial shrub native to South America that belongs to the plant family Solanaceae. Records indicate that this plant was initially detected in Glades County, FL, in 1988. TSA primarily invades pastures, fields, and parks, but also has the potential to invade open forest and other natural areas. TSA forms thick stands that can be impenetrable to livestock, large wildlife, and humans. Livestock, deer, raccoons, and other wildlife eat the mature fruit and spread the seed in their manure. TSA also serves as a reservoir for various diseases and insect pests of solanaceous crops. Annual control costs have been estimated at $6.5 million to $16 million. It was placed on the Federal Noxious Weed List in 1995 and is listed as one of the most invasive species in Florida by the Florida Exotic Pest Plant Council.

In 2002, PPQ's Eastern Region began supporting efforts to develop and implement classical biological control for TSA that were underway at the University of Florida-Gainesville, with CPHST providing technical oversight of the project. A permit to field release the South American leaf-feeding beetle *Gratiana boliviana* was approved in 2003. The beetle has since been introduced into Florida (2003), Alabama and Georgia (2004), and Texas (2007) through a collaborative effort by individuals from universities as well as State and Federal agencies in those four States. These collaborators developed methods to mass rear, release, and evaluate the biological control agent. More than 200,000 beetles have been released in over 350 locations. This technology was transferred through trade and scientific journal publications and YouTube videos to extension agents, cattle ranchers, conservation organizations, and others as part of an integrated management approach to controlling TSA.

In 2009, a multi-agency, statewide survey in Florida was conducted to determine the distribution of *G. boliviana*. The survey revealed that the beetle was spreading on its own and that no further releases were required in south and central Florida, but that it was not well-established in north Florida. In pastures and natural areas in central and south Florida, the beetle has resulted in substantial suppression of TSA density and fruit production. This was corroborated in a preliminary survey/economic analysis conducted by Florida A&M University in 2010, which indicated that ranchers no longer consider the weed a pest in many of these areas and that cost for control has decreased significantly.

In Alabama and Georgia, CPHST Miami and ARS Tallahassee collaborated to determine if the beetle could overwinter in these more northern climates and if field insectaries could

be established. Although the studies showed that the beetle technically can survive winters in Georgia and Alabama, the data generally paralleled those from Florida that indicated the beetle is having less of an impact in northern counties. For example, at the most northern location near Montgomery, AL, the beetles survived through two winters that had periodic snowfalls. However, the population remained very low and eventually disappeared. Similarly, *G. boliviana* was found to survive but not thrive in field cages in east Texas, where winter temperatures dropped to near 30 ºF. Currently, it is unclear if the beetle's poor performance in more northern climates is because the beetle does less well in cooler climates, the plant does less well in cooler climates, or more aggressive management practices were utilized in the north to eradicate the plant. The group will conduct controlled field studies in 2011 at three different latitudes in Florida to try and determine what factors are limiting *G. boliviana*'s success.

CPHST Miami also worked with University of Florida scientists to test the host range of a second leaf-feeding beetle, *Gratiana graminea*, which is a biological control agent of TSA that is more tolerant of low temperatures. In quarantine studies, the beetle fed and developed on Thai variety eggplant (*Solanum melongena*), wild turkey berry (*Solanum torvum*), and TSA. This information was provided to the Technical Advisory Group for Biological Control Agents of Weeds to help determine the risk of releasing this biological control agent in the field.

Guatemala Station - Operations Support for Fruit Fly Preventative Release and Eradication Programs - Moscamed Gradual Advance Plan (GAP)

Location: Guatemala, CPHST Fruit Fly Unit
Lead Scientist: Pedro Rendon
Team Members: Carlos Caceras, Felipe Geronimo, Aracely De Galen, Mike Stefan, APHIS IS Guatemala, Ministry of Agriculture Guatemala & Mexico, Moscamed Program

The Mediterranean fruit fly (Medfly) is a serious pest that threatens the United States by potential expansion of its range from Central America into Mexico and northward. The Moscamed program, on the border between Guatemala and Mexico, has maintained a barrier to this northward expansion for the last 30 years. This pest barrier program has at times failed, and the pest has threatened to push further into areas of Mexico and northward into the United States. This event would be a disaster to agricultural production in Mexico and the United States. The Moscamed program has developed a Medfly control strategy known as the Gradual Advance Plan (GAP). CPHST Guatemala has played an integral role in developing the technical strategy that has helped the GAP become a successful component of the Moscamed Barrier. The GAP, as well as the improved methods technology, has allowed the program to push the barrier approximately 100 kilometers away from the historic barrier on the Guatemala and Mexican borders even while the program budget decreased. This has reduced the pest pressure on Mexico and greatly strengthened the overall program effort.

Key contributions of CPHST Guatemala to the GAP include:

- Development of female traps and lures to better predict Medfly population dynamics.
- Ecological evaluations employing geographic information systems technology to determine where the Medfly "hot spots" occur and to allow new control strategies that knock down the pest in these areas before they migrate and spread.
- Identification of coffee "Beneficios" or processing centers as high-risk sites requiring targeted control of the Medfly, and their subsequent use as sentinel sites for detection purposes.
- Development of environmentally safe pesticide formulations that significantly reduce the amount of pesticide use and are generally accepted by the public.
- Development of novel bait stations that can be used in environmentally sensitive areas where aerial sprays are not allowed.
- Combining the use of parasitic wasps that provide biological control with the sterile release of Medflies to provide enhanced control.
- Development of improved Medfly colony strains and rearing processes that increase production and allow for areawide sterile releases that keep the areas "free" of the pest post-treatment.
- Development of an environmental monitoring program that assesses the biological impact of the sprays to gain the support of the very important beekeeping industry.

The CPHST Guatemala fruit fly team has been a key component in the recent success within this difficult and challenging Moscamed program area. Many of the same technologies are also being transferred to the U.S. domestic fruit fly programs to improve efficiency, maximize resources, and protect the ag-

ricultural industry from incursions of these pests from other areas of the world.

Evaluation of the Sterile Insect Technique for Light Brown Apple Moth Control

Location: CPHST Light Brown Apple Moth Unit
Lead Scientist: Greg Simmons
Team Members: Alex Cunningham, Derrick Hammons, Leslie Foss, Stephen Friedt, Tom Greene, Katie Harding, Saben Kane, Luis Mazuera, Amber Reece, Meghan Brumgard, Patrick Mingus
Cooperators: Nada Carruthers, Max Suckling, Bill Woods, Don McGuiness, Eric Jang

LBAM has recently invaded Northern California and become established in 18 counties within the State. LBAM poses a threat to agricultural crops, including grapes, citrus, pome fruits, stone fruits, and berry crops, and to the ornamental nursery industry. LBAM also infests a large number of native and forest plant species.

PPQ and the California Department of Food and Agriculture are working on a cooperative program to combat this pest using an integrated pest management approach. The SIT will be a key component of the overall control program for LBAM in California. The SIT has been successful against several major program pests, including Medfly, the Mexican fruit fly, screwworm, and several moth species such as PBW, codling moth, and painted apple moth. Several insect control tools are complementary with the SIT, including mating disruption, biological control, and biorational pesticides.

To develop the SIT as an operational tool for LBAM control, PPQ has begun a project to evaluate the use of the SIT for LBAM. The project's goals are to develop and adapt mass-rearing, irradiation, handling, release, and evaluation technologies to lay the groundwork for future implementation of an operational SIT program.

During the last year, the Moss Landing rearing facility was established and made operational. The facility's capacity is sufficient to produce an estimated 500,000 moths per week, though so far the highest production achieved has been 100,000 moths per week. Over the last season, production ranged from 50,000 to 100,000 moths per week and 1 million to 1.5 million eggs per week. Methods development testing continues to refine the rearing system to support higher production of high-quality moths. Testing in the last year focused on improvements to the egg-laying cages, larval rearing, and adult collection.

Additional work was conducted in the laboratory to determine the optimal radiation dose for LBAM resulting in high-quality sterile moths. Radiation biology studies were conducted in California and by cooperators in Australia, Hawaii, and New Zealand. These studies have shown that a radiation dose of 300 Gray will achieve effective sterility and acceptable moth quality. Work is ongoing to determine if a lower dose could be used to improve moth quality and allow the use of an inherited sterility strategy. So far, this work suggests that 250 Gray would work for this strategy, which may be suitable to combat LBAM in high-population areas.

Field testing of sterile LBAM to develop SIT methods began in California vineyards in May 2010 and was completed in mid-November of 2010. A total of 15 separate field releases were made in 2 vineyards in Napa and Sonoma for a total release of 65,000 sterile moths. The releases were made at the center of a grid of pheromone-monitoring traps. These releases were designed to determine how many and how often sterile moths need to be released by estimating how far the moths could fly, how long they live after field release, and the rate at which they move.

For the 15 releases, the highest recapture rate for male moths was 6.1 percent after a single release. Active moths were observed in the field up to 15 days after release, with the most moths being recaptured in the first 8 days after release. With this field longevity, a 3-day release interval would be adequate to build up the moth population in a field. The longest movement observed was 75 meters, suggesting that male moths are capable of flying at least that far.

A full season of SIT release evaluations (funded by a cooperative agreement with PPQ) was completed in New Zealand in April 2010. This test was conducted in a vineyard in an area where LBAM is well-established, allowing the measurement of sterile moth interactions with wild moths. Recapture rates for sterile moths were equivalent to the releases in California, and high recapture rates of sterile moths relative to control moths suggested that the effects of radiation would not significantly impact the quality of released sterile moths. Also, in 2010, the first test release of sterile moths against a fertile population was conducted. In a single release in large field cages using a 10-to-1 over-flooding ratio, the fertility of re-

leased wild moths was reduced by an average of 82 percent relative to fertile moth release control cages.

Features of the Newly Launched Fumigation Form 429 Database

Location: Treatment Quality Assurance Unit
Lead Scientist: Laura Jeffers
Team Members: Scott Wood, Woodward Bailey, Leah Floyd
Cooperators: Karl Suiter (NSF Center for Integrated Pest Management)

On October 12, 2010, the Treatment Quality Assurance Unit (TQAU) launched a new version of the Fumigation Form 429 Database. One of several databases in the Commodity Treatment Information System, the Fumigation Form 429 Database collects treatment data from PPQ-monitored fumigations in the United States. The Commodity Treatment Information System (CTIS; https://treatments.cphst.org/tqau/) is a secure, online Web-enabled data system that collects, stores, and creates reports from phytosanitary treatments and provides regulatory information. These systems are critical to supporting phytosanitary treatments around the world.

New features of the Fumigation Form 429 Database:

- Reporting stations are customized to include information such as fumigation location, contractor, enclosure type, and substrate.
- Cargo items can be identified for import, export, domestic, or import and re-export quarantine fumigations. Originally, the database was designed to accommodate import fumigation data only.
- Several measures are in place to reduce data entry error. For instance, officers cannot enter fumigant usage values that are 10-percent greater or less than the amount calculated by the database. This prevents over-reporting or under-reporting of fumigants to the EPA.
- New statistical and search functions allow retrieval of previously unavailable information about pest taxa, fumigation locations, fumigation contractors, treatment schedules, and treatment adjustments.
- All treatment schedule parameters are linked. If a treatment schedule is selected from the drop-down list, only the temperature ranges available for that schedule appear in the next drop-down list. When a temperature range is selected, only the dosages for that temperature range appear in the next drop-down list.

The most successful additions to the database are the quality assurance features, specifically the CT Product Alerts. The efficacy of a treatment can be quantified using a value called the concentration x time product (or CT product). For a fumigation to be successful (i.e., provide control of the target pest), the CT $product_{fumigation}$ must be more than or equal to the CT $product_{schedule}$. A CT $product_{schedule}$ was calculated for every methyl bromide treatment schedule in the PPQ Treatment Manual and entered into the database. After an officer submits a fumigation report to the database, a CT $product_{fumigation}$ is auto-generated and compared to a CT $product_{schedule}$. If the CT $product_{fumigation}$ is less than the CT $product_{schedule}$, a notification is sent to TQAU, PPQ's Eastern Region, and PPQ's Western Region. If the error is not caught by the supervisor, a second notification is sent to TQAU, PPQ's Eastern Region, and PPQ's Western Region when the report is approved.

Reasons for CT Product Alerts:

- **Treatment Failure.** The fumigation was performed as required by the PPQ Treatment Manual, but the appropriate CT product was not reached. This situation would require a change to the treatment schedule.
- **Operational Failure.** The fumigation was not performed as required by the PPQ Treatment Manual. No change to the treatment schedule is necessary. This error can result if the schedule minimums are not reached, gas concentrations differ more than 4 ounces per 1,000 ft^3, additional gas and/or time was not added correctly, or monitoring leads are blocked.
- **Entry Error.** The fumigation data is entered into the database incorrectly. Usually, this error results from missing concentration readings or incorrectly entered treatment parameters.

Since their inception in October 2010, several hundred alerts have been sent to TQAU and regional staff. Approximately 70 percent of the notifications are due to data entry errors, while operational and treatment failures account for the remaining 30 percent.

Prior to the launch of the new version of the database, TQAU completed a series of instructional workshops to familiarize users in PPQ's Eastern Region, Western Region, and headquarters offices with the new features of the database. Additionally, members of the PPQ Professional Development Center were invited to attend the instructional workshops. The Professional Development Center will assume responsibility for routine database training during their fumigation

workshops, which are held semi-annually. All workshops were delivered via Webinar, using audio and Internet conferencing. An estimated 250 people were trained across the United States, which saved TQAU a substantial amount of travel money over face-to-face training.

Publications

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Scientific Meetings

CPHST scientists give presentations and attend national and international scientific meetings, workshops, and conferences in order to promote the exchange of the latest scientific information on plant protection. The following is a list of such meetings attended in 2010.

- American Chemical Society National Meeting
- American Phytopathological Society Annual Meeting
- Asia-Pacific Association of Chemical Ecologists Meeting
- Association of Analytical Communities International Annual Meeting
- Global Biosecurity 2010
- California Oak Mortality Task Force Annual Meeting
- Caribbean Food Crops Society Annual Meeting
- Emerald Ash Borer Research and Development Meeting
- Entomological Society of America Annual Meeting
- ESRI International Geographic Information Systems User Conference
- European Food Safety Authority, Science Supporting Risk Surveillance of Imports
- FAO/IAEA Research Co-ordination Meeting
- Florida Entomological Society Meeting
- Florida Pesticide Residue Workshop
- Foreign Plant Germplasm Diagnostics ARS/APHIS Annual Meeting
- Gypsy Moth Review
- Imported Fire Ant Research Annual Conference
- Integrated Consortium of Laboratory Networks Annual Meeting
- International Acarology Congress
- International Cotton Pest Work Committee
- International Huanglongbing/Zebra Chip Meeting
- International Invasive Ant Management Workshop
- International Organization of Citrus Virologists Conference
- International Pest Risk Modeling and Mapping Workshop
- International *Plum Pox Virus* Symposium 2010
- International Symposium on Fruit Flies of Economic Importance
- JMP Discovery Summit
- Methyl Bromide Alternatives Outreach Meeting
- Midwest Weather Working Group Annual Meeting
- National Cooperative Agricultural Pest Survey Conference
- National Citrus Research Coordination Symposium
- National Cotton Council
- National Grasshopper Management Board Annual Meeting
- National Plant Diagnostic Network Annual Meeting
- National Symposium on Microbial Biocontrol of Arthropods, Weeds, and Plant Pathogens
- NCERA-213 Annual Meeting
- New Technologies Conference for Citrus
- North American Plant Protection Organization Annual Meeting
- Ornamental Workshop on Diseases and Pests
- PC-HYSPLIT Workshop
- Plantwise Workshop
- Rocky Mountain National Park Research Conference
- Quadrilateral Science Collaboration Diagnostic Tools Collaboration Project
- Quadrilateral Science Collaboration Methyl Bromide Alternatives Project
- Southern Forest Insect Work Conference
- Subtropical Plant Science Society
- Tri-Lateral Port Monitoring Program Meeting
- Tri-National Citrus Health Response Program Meeting
- University of Alabama-Huntsville Bioretreat
- U.S./Canada *Plum Pox Virus* Update and International Research Symposium
- USDA-FS Eastern Forest Environmental Threat Assessment Center Meeting
- USDA APHIS/CDC Select Agent Program Workshop
- USDA Interagency Research Forum on Invasive Species
- USDA-APHIS International Workshop on *Tamarixia* Species
- W-2185 Regional Biological Control Project
- Weed Science Society of America Annual Meeting
- Weeds Across the Borders
- WERA-89 Potato Virus Disease Control Committee Annual Meeting
- Western Gypsy Moth Meeting

Appendix A. List of International Partners/Collaborators

International partners and collaborators provide support for safeguarding our borders from invasive plant pests. CPHST collaborated with 33 countries on national and global projects.

Australia
Austria
Belize
Bulgaria
Barbados
Belgium
Brazil
Canada
China
Costa Rica
Colombia
Czech Republic
Dominican Republic
Egypt
Ethiopia
Federated States of Micronesia
Ghana
Guam
Guatemala
Kenya
Malaysia
Mexico
Morocco
New Zealand
Palau
Panama
Romania
Serbia
Switzerland
Spain
Tunisia
United Kingdom
Ukraine

Appendix B. Funded Projects for Fiscal Year 2010

Funding Category	$ in thousands
AQI-User Fee	12,643
Farm Bill	8,363
Plant Methods	7,815
Biocontrol	3,485
Light Brown Apple Moth	3,200
Emerald Ash Borer	2,431
Pest Detection	1,196
Miscellaneous Pests	1,000
Sirex	845
Fruit Fly Exclusion and Detection	618
Grasshopper	500
Sudden Oak Death	250
Imported Fire Ant	214
Select Agents	150
Pale Cyst Nematode	104
Boll Weevil	100
Gypsy Moth	75
Noxious Weeds	45
Golden Nematode	20
Total	**44,241**

Funding lines for CPHST in fiscal year 2010. Includes allocated funds of $35.9 million and Farm Bill Section 10201 funds of about $8.4 million.

Beltsville Laboratory

- Validation of conventional and real-time polymerase chain reaction (PCR) diagnostics for rapid onsite detection of potato wart
- Development of potyvirus group-specific PCR diagnostic assays for grasses and sweet potatoes for viruses infecting imported germplasm
- Development of PCR diagnostic assays for specific viruses infecting imported germplasm
- Development of advanced diagnostics for *Plum pox virus* (PPV) and final characterization of PPV strain W detected in Ukrainian *Prunus* interception
- Adaptation of Cellular Analysis and Notification of Antigen Risk and Yield (CANARY) biosensors for the rapid detection of regulated plant pathogens
- Development of proficiency test reagents for high-consequence plant pathogens for delivery to the National Plant Protection Laboratory Accreditation Program (NPPLAP) for laboratory certification Huanglongbing (HLB), *P. ramorum*, and PPV
- Validation of conventional and real-time PCR assays for citrus black spot
- Development and validation of conventional and real-time PCR assays for sweet orange scab

Farm Bill-Funded Projects

- Development of a lateral flow microarray for citrus canker, citrus variegated chlorosis, citrus greening, and PPV (Mesa Tech)
- Evaluation of Surface Plasmon Resonance Technology (Rutgers University)
- Evaluate Optical Genomic Mapping Technology (Opgen)
- Rust Barcoding (Louisiana State University)
- Development of CANARY B-cell lines (Massachusetts Institute of Technology)
- Adaptation of a real-time PCR for the detection of the citrus variegated chlorosis strain of *Xylella fastidiosa*
- Validation of PCR diagnostics methods for the adaptation and validation of PCR detection methods for *Citrus leprosis virus* (CiLV)
- Sampling the HLB genome for improved detection and identification of the HLB causal bacterium
- Development of CiLV polyclonal and monoclonal antibodies (University of Florida)
- Monoclonal antibody development for CiLV, citrus variegated chlorosis, and HLB (USDA-ARS)
- Hands-on laboratory training of National Plant Disease Network diagnosticians (USDA-National Institute of Food and Agriculture)
- Quadrilateral Scientific Cooperation for Plant Biosecurity: Diagnostic Tools Collaboration work group for exchange and cooperation of molecular and biochemical diagnostic assays (Australia, New Zealand, and Canada)

Gulfport Laboratory

Chemistry Methods Development Projects

- New lure methods development: extraction, instrumental analysis, and/or emission rate studies for Linneatin lure, BW Grandlure, Khapra beetle lure, and light brown apple moth (LBAM)
- Chlor-Tetracycline in insects for CPHST-Phoenix
- Diflubenzuron in vegetation "Quick, Easy, Cheap, Effective, Rugged, and Safe" (QuEChERS) method adaptations
- Liquid chromatography/mass spectrometry (LC/MS) emamectin benzoate methods development and analysis

- Carbaryl in vegetation and soil methods development
- Grasshopper program field spray mix studies
- Methods development for imidacloprid in bees, honey, and wax
- Z-Nose use in identification of signature compounds

Imported Fire Ant (IFA) Projects

- Biological control of the IFA using phorid flies: cooperative rearing and release program
- Biological control of the IFA: monitoring of field releases of *Thelohania solenopsae* and *Pseudacteon* spp.
- Grass sod and bait treatments for control of IFA (University of Arkansas)
- Development of quarantine treatments for field grown/balled-and-burlapped nursery stock
- New treatments for containerized nursery stock

Farm Bill-Funded Projects

- Methods development for extraction and analytical process for the multicomponent spruce blend lure and *P. chalcographus* lure (University of Alabama)
- Development of a model to determine the origin of mangoes using isotope analysis (DHS Customs and Border Protection)
- Development of rapid assay kit to identify IFA from other fire ants and to develop a species-specific IFA trap (USDA-Agricultural Research Service [ARS])

Fort Collins Laboratory

Identification Tools

- Federal Noxious Weed Disseminules of the U.S., Edition 2.1 (CDFA)
- An Identification Tool for Bark Beetles of the Southeastern United States (North Carolina State University, Southern Plant Diagnostic Network, Oregon Department of Agriculture)
- Xyleborini Ambrosia Beetles – An Identification Tool to the World Genera (University of Wisconsin, Michigan State University, North Carolina State University, University of California)
- Bark Beetle Genera of the United States (Colorado State University, USDA's Forest Service)
- ID Source (Colorado State University)
- Resource to Commodity-based tools to Central Valley Table Grapes
- Screening Aid for Spiders of California Central Valley Table Grape Production Areas (University of California-Riverside)
- Screening Aid for Weed Disseminules of California Central Valley Table Grape Production Areas (California Department of Food and Agriculture [CDFA])
- TortAI: Tortricids of Agricultural Importance (Colorado State University, CDFA)
- PBW ID: Screening Tools for Pink Bollworm (University of Arizona)
- Common Nymphal Grasshoppers of the Western United States
- Grasshoppers of the Western United States, Edition 4 (Chadron State College, University of Nebraska)
- Identification Resource for the Fruit Fly Species of *Anastrepha* and *Toxotrypana* (USDA-ARS, Universidad de Panama, Universidade de Sao Paulo)

Farm Bill-Funded Identification Tools

- Flat Mites of the World: Identification Tool to Genera and Critical Species (University of Maryland, USDA-ARS, Australia Queensland Museum)
- Dried Botanical Identification Tool (Delaware State University)
- A Resource for Pests and Diseases of Cultivated Citrus in the United States (University of Florida, Southern Plant Diagnostic Network, North Carolina State University, University of California-Riverside)
- A Resource for Pests and Diseases of Cultivated Palms (University of Florida, Florida A&M University, Florida Division of Plant Industry, North Carolina State University)
- Wood-Boring Beetles of the World (University of New Mexico, Australia CSIRO, USDA-ARS, University of Maryland)
- Interactive Identification Tool to Polyphagous and Cosmopolitan Aphids (USDA-ARS, University of Maryland)
- Identification Tool to Molluscs of Agricultural Significance to the United States (University of Florida)
- Invasive Ants of the United States (University of Illinois)

Biological Control

- Technology transfer and information services in weed management and biological control
- Development of biological control for management of dyer's woad (CABI Europe-Switzerland)
- Development of biological control for management of perennial pepperweed (CABI Europe-Switzerland)
- Development of biological control for management of hoary cress (CABI Europe-Switzerland)

- Development of biological control for management of garlic mustard (CABI Europe-Switzerland)
- Development and delivery of biological control for management of yellow toadflax (CABI Europe-Switzerland, Colorado State University)
- Development of biological control for management of hound's-tongue (CABI Europe-Switzerland)
- Development of biological control for management of field bindweed (CABI Europe-Switzerland)
- Development of biological control for management of orange hawkweed (CABI Europe-Switzerland)
- Development and delivery of biological control for management of Russian knapweed (CABI Europe-Switzerland, Montana State University)
- Risk assessment and monitoring of target and nontarget plant utilization by the hound's-tongue root weevil (University of Idaho)
- Biological control of saltcedars
- Assessment of endemic arthropods for biological control of Canada thistle (Belgrade University)
- Greenhouse-based rearing program for two weed biocontrol agents, *Jaapiella ivannikovi* and *Mecinus janthinus*
- Biological control of LBAM (CDFA)
- Development of rearing systems for beneficial root feeders (Bureau of Land Management and Nez Perce Bio-Control Center)
- Addressing the problem of mass-produced pink bollworm reared on the red diet lacking red coloration
- Survey for natural enemies of Canada thistle (CABI United Kingdom)
- Survey for natural enemies of perennial pepperweed

Weed Control

- Benghal dayflower seedbank control with five soil fumigants
- Common tansy control with two sprayer systems
- Use of global positioning system and geographic information systems technology to estimate patch spread rate for several invasive plant species
- Giant hogweed control with two Milestone formulations
- Agricultural waste disposal project involving microbial efficacy tests with a chemical digester and a shredder/steamer system (Farm Bill-funded)
- Developing guidelines for the molecular identification of invasive plants
- Development of molecular diagnostic tools to identify five invasive plants (University of Alabama)
- Enhancing the transfer of technical methods for the identification of invasive plants through video documentation (University of Alabama)
- Pathway analyses of *Mikania micrantha* using DNA sequences (University of Florida)
- Methods development to evaluate the potential of plant species to become invasive and identify invasive plants (Colorado State University)
- Potential for a widespread horticultural cultivar to breed with a Federal noxious weed (University of Florida)

Survey Support

- Development of commodity-based survey documents for the Cooperative Agricultural Pest Survey (CAPS) community
- Commodity reference and survey guidelines for exotic pests of corn
- Commodity reference and survey guidelines for exotic pests of stone fruit (West Virginia University)
- Commodity reference and survey guidelines for exotic pests of cotton (University of Arizona)
- CAPS-approved survey and diagnostic methods development
- Risk assessments for exotic pests of woody plants

Spatial Technologies

- Modeling pest spread based on landscape connectivity concepts
- Enhancing Asian gypsy moth trapping in California
- Evaluating global weather patterns and their relation to grasshoppers
- Modeling pest spread based on landscape connectivity concepts
- Providing geospatial expertise for data management and analysis to the Mission Lab's Asian citrus psyllid (ACP) monitoring study
- Comparison of CLIMEX and North Carolina State University/APHIS Plant Pest Forecasting System (NAPPFAST) models for LBAM establishment
- Update of HOPPER Expert System to include economic analysis
- Monitoring control strategies for ACP in Texas
- Correlations between ethnic population centers and recurring fruit fly introductions in California

Mission Laboratory

Fruit Fly Program Support

- Mexican fruit fly (Mexfly) diet formulation
- Mexfly new strain development
- Assessment, identification, and suppression of fungal contaminants in the Mexfly mass-rearing facility
- Field assessment of combined releases of sterile insect technique (SIT) flies and parasitoids for controlling fruit flies (Guatemala)
- Support for Florida tephritid fruit fly preventative release and eradication programs
- Assessing detection sensitivity of the California and Florida sampling grid to *Bactrocera cucurbitae* and *B. dorsalis*

Molecular Diagnostics

- Develop molecular diagnostic techniques that identify foreign fruit fly pests
- Develop molecular techniques that identify cryptic thrip species
- Development of molecular diagnostics for Asian long-horned beetle
- Molecular genetic assessment of *Beddingia* (*Delandenus*) *siricidicola* populations
- Development of molecular diagnostic techniques for mollusks of economic importance
- Improved molecular diagnostic methods for identification of immature LBAM
- Development of a diagnostic tool for identifying the geographic source of intercepted Dacine fruit flies

Integrated Pest Management

- Operation of the CPHST Arthropod Quarantine facility
- Develop classical biological control methods for Asian cycad scale
- Developing integrated pest management strategies for *Crypticerya genistae*, an invasive scale pest in Puerto Rico
- Developing biological control strategies for Harrisia cactus mealybug
- Efficacy of packinghouse procedures on the mitigation of armored scales on Hass avocado

Farm Bill-Funded Projects

- DNA barcoding of woodborers (Purdue University)
- Evaluating the biological control of ACP in the Rio Grande Valley of Texas
- Citrus canker epidemiology studies
- CiLV identification and monitoring of mite populations in Texas citrus
- Studies in HLB management through inter-planting citrus with guava
- A pilot project: areawide management of ACP in Texas
- Effect of heat and cold on activity of canker lesions on citrus fruit (University of Florida)
- Treatments for ACP control in organic citrus production (Texas A&M-Kingsville)

Otis Laboratory

Forest Pests Program Support

- Support for the Russian/Japanese/Chinese/Korean Exotic *Lymantria* exclusion program (USDA-Foreign Agricultural Service [FAS])
- Forecasting pest potential through offshore assessments (primarily Asian defoliators) (USDA-FAS)
- Molecular analysis of male gypsy moths trapped in U.S. ports and other high-risk areas
- Asian gypsy moth research (Korea, APHIS' International Services)

Biological Control

- Production of insect diets and life stages for use in APHIS and other cooperative research programs

Phytosanitary Treatments

- Alternative quarantine treatments for invasive hitchhiking snails
- Fumigation and heat for control of wood-boring insects in pallet wood/solid wood-packing material to meet IPPC request
- Phosphine gas in combination with sustained cold treatment and gamma radiation as a methyl bromide alternative for imported fruits

Pest Survey and Detection Programs

- Odor-based detection and monitoring systems for exotic pests
- Survey, detection, and treatment evaluation on insects associated with wood-packaging materials from China
- Improved analysis and interpretation of insect trapping data
- Production of lures for European grapevine moth program

Emerald Ash Borer (EAB) Methods Development

- Infestation dynamics of ALB and EAB in North America
- Evaluation of preference and suitability of tree species as hosts for exotic wood-boring beetles
- Evaluation of soil applications of insecticides for control of exotic wood-boring beetles
- Biological control strategies for EAB
- Evaluation of systemic insecticides for woodborer control (China, USDA-FAS)
- Distribution of EAB in China (China, USDA-FAS)
- Fumigation of wood as a phytosanitary treatment for EAB
- Behavior, biology, and control of exotic wood-boring beetles (University of Massachusetts)
- Insecticide testing for control of exotic woodborers
- Pesticide residue determination for systemic insecticides in trees
- Ecology of EAB in its native habitat
- Quarantine treatments for EAB
- Development of methods for detecting low-level populations of EAB (Michigan Technological University)
- Semiochemicals for exotic wood-boring beetles (VMI)
- Assessing the efficacy of mobile *Cerceris fumipennis* colonies (USDA-Forest Service)
- Management and ecology of EAB (University of Maryland)
- Development of survey tools for EAB

ALB Support

- Improved rearing for ALB
- Molecular and genetic characterization of ALB (China, USDA-FAS)
- Evaluation of insecticides for control of ALB (China, USDA-FAS)

Sirex Woodwasp Support

- Development of monitoring tools for *Sirex*
- Biological control of *Sirex*
- Validation of phytosanitary treatment options for *Sirex*
- Development of degree day model for *Sirex*
- Behavior and sensory ecology of *Sirex*
- Semiochemical attractants for *Sirex* (Pennsylvania State University)
- Host-based attractants for *Sirex* (North Carolina State University)
- Biological control methods for *Sirex* (Cornell University)

LBAM Support

- Alternative regulatory treatments for LBAM
- Improved trapping methods for LBAM
- Optimize mating disruption for LBAM (New Zealand)
- Production of lures for LBAM national survey
- Improved pheromone formulations for LBAM (University of California-Riverside)
- Assess insecticides for LBAM
- Populations dynamics/ecology for LBAM (University of California-Davis)

Farm Bill-Funded Projects

- Development of an aggregation pheromone attractant for the walnut twig borer (University of California-Davis)
- Development and implementation of a pheromone-baited trap for the walnut twig beetle (USDA-Forest Service)
- Monitoring and control of European grapevine moth (University of California-Davis)
- Development of pheromone-based lures for Lepidoptera
- Development of pheromone-based survey methods for the fruit-piercing moth (USDA-ARS)
- Development of trapping methods for exotic wood-boring beetles (*Buprestidae*) (Pennsylvania State University)
- Assessment and development of pheromonal attractants for exotic longhorned beetles (University of California-Riverside)
- Assessment of trapping methods for exotic wood-boring beetles in Canada and Europe (USDA-Forest Service and Canada)
- Novel approaches to attraction-based surveys for exotic wood-boring beetles (Tennessee State University)

Phoenix Laboratory

Rangeland Grasshopper/Mormon Cricket

- CPHST and ARS cooperative cage studies to develop superior microbial control agents for Mormon cricket and grasshopper control
- Discovery, identification, and biological characterization of new fungi with potential for insect control isolated from U.S. soil in an effective Federal/State/academic collaborative effort
- Survey of untreated field-collected grasshopper cadavers for prevalence of naturally occurring pathogenic fungi

- Field evaluation of aerially applied Coragen® for effectiveness against grasshoppers on rangeland
- Evaluation of ground application methods of diflubenzuron for control of rangeland grasshoppers
- Evaluation of candidate toxicant replacement for carbaryl in grasshopper and Mormon cricket baits
- Analysis of biopesticides used for control of rangeland grasshopper in northern Utah
- Simplifying diluents used in aerially applied, low-volume diflubenzuron sprays against rangeland grasshoppers
- Initial comparison of the standard organophosphate treatment, malathion, and a pyrethroid, B cyfluthrin, applied at the label rates and lower rates for control of rangeland grasshoppers in South Dakota
- Comparison of the standard APHIS 80º flat fan nozzle with a 40º flat fan nozzle for delivery of diflubenzuron for rangeland grasshopper control in South Dakota

Pink Bollworm (PBW)

- Optimize use of 34 millimeter extruder as backup for the large extruder and to make small batches of research diets
- Investigations for definitive identification methods for APHIS mass-reared PBW
- Testing genetically modified PBW (OX3402CC) functionality
- Measuring diapause in PBW rearing facility population
- Pheromone mating disruption for PBW: refinements to the carrier in a sprayable formulation and aerial application by helicopter
- Comparison of mating competence in genetically modified female PBW (OX1138BB) versus APHIS mass-reared moths
- Field tests to evaluate alternative (non-aerial) means to release sterile, mass-reared PBW post-eradication
- Evaluation of PBW response to freeze-dried/reconstituted diet in an effort to save shipping and cold storage costs
- Administration of two cooperative agreements to support the International PBW Eradication Program
- Development and distribution of a comprehensive PBW bibliography to cooperators to enhance research efforts
- Evaluation of a promising, non-carcinogenic egg treatment to replace formalin in the PBW rearing facility
- Demonstration of PBW development on okra, an alternate host

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

- Through the New Pest Advisory Group, assess new and imminent pest introductions into the United States and make recommendations to PPQ management regarding appropriate agency responses to exotic plant pests
- Perform deregulation evaluations for established pests to support policy decisions on pest status for consistency with import actions

Accreditation and Certification of Risk Analysis Functions

- Through audits and improvements, maintain ISO certification for the Lab's commodity risk assessments and New Pest Advisory Group

Outreach and Training/Capacity Building/Regulatory Curricula

- Provide instructors for a regulatory science minor at North Carolina State University
- Maintain the strong cooperative relationship established between CPHST and 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 APHIS' decisionmaking 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: International Plant Protection Convention, North American Plant Protection Organization

- Lend support, time, and expertise to international organizations by participating on international working groups to write standards and review draft standards and the specifications for new standards as they become available
- Manage and maintain the Web site for the Phytosanitary Alert System Panel, which provides oversight to early warning initiatives for the North American Plant Protection Organization

Information Systems and Biosurveillance Analysis Forecasting

- Maintain and expand the Global Pest and Disease Database
- Through the NAPPFAST, 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 NAPPFAST 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 notifications

Weed Risk Assessment

- Generate model and pest list for weeds
- In support of the Q-37 revision, revise weed risk assessment guidelines to improve and streamline the process
- Validate the risk assessment model and compare its accuracy with that of the Australian weed risk assessment model that is being used elsewhere
- 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

Reference Management

- Maintain and improve physical library (currently 4,000 scientific references, including publications, large scale maps, and videos)
- Maintain and improve the pest risk analysis (PRA) library in Endnote (currently 600 PRA that are indexed and searchable)
- Maintain and improve the digital library of scientific references (currently 14,000 documents 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)

Biocontrol Unit

A full listing of the names, project titles, and publications of CPHST scientists working on biocontrol can be found under the highlights for the individual CPHST laboratories, with the exceptions of Amy Roda and Scott Weihman at the Miami Station as well as Pedro Rendón and Carlos Cáceres at the Guatemala Station, who are administered from the Director's Office in Raleigh, NC.

Miami Station

- Biological control of tropical soda apple
- Biological control of coffee mealybug *P. lilacinus*
- Developing new pest response guidelines for red palm weevil
- Assessing biological control options for Harrisia cactus mealybug in Florida
- Developing biological control and survey technologies for the Pacific mealybug *P. minor*

- Improving techniques for detecting prohibited plants and invasive insects at ports of entry

Guatemala Station

- Developing mass-rearing techniques for the Mediterranean fruit fly (Medfly) egg parasitoid *Fopius ceratitivorous*
- Field testing augmentative releases of parasitoids for Medfly control
- Rearing olive fly parasitoids in support of classical biological control of olive fly in California

Fruit Fly Unit

These projects include fruit fly-related work conducted at multiple locations across CPHST.

Operations Support for Fruit Fly Preventative Release and Eradication Programs

- Evaluation of fruit fly diets
- Larval collections systems
- Pupation media and dispensing
- Cryopreservation
- Irradiation dose calibration
- Fruit fly strain formation
- *Anastrepha* support and cost-efficient mass-rearing
- *Anastrepha* field quality control assessment
- Diets for Mexfly
- Release center support
- Operational validation of ginger root oil
- Field evaluation of mating competitiveness of sterile insects
- Adult diets and agar–semi liquids for emergence towers

Research and Development Support

- Alternate hosts identification
- Climatic factors that influence pest populations

Field Control Activities

- Coffee processing centers and Medfly populations
- Field validations of differing rates of GF-120 baits
- Evaluation of SPLAT lure
- Design and evaluation of bait stations
- Trap and lure evaluations for research and procurement
- SIT and parasitoids
- Genetically engineered strains
- Classical Mexfly genetic sexing development
- Mexfly diet development/rearing support
- Mexfly new strain development
- Implementations of egg bubbling system
- Thermodynamics of Mexfly rearing
- Evaluation of new fruit fly trap/lure trapping agent combinations
- Lower Rio Grande Valley Mexfly eradication support
- Suppression of fungal contaminants in Mexfly rearing
- Development/evaluations of DNA barcodes databases for fruit fly pests
- Evaluation of utility of museum collections for DNA barcode studies
- Conduct/facilitate collecting of fruit flies for DNA molecular work
- Development of DNA sequence databases based on broad-scale genomic variation
- Evaluation of genetic diversity in Medfly sterile insect technique world labs
- Development of multigene pathway analysis database for Medfly
- Evaluation of population structure within the Mexfly and West Indian fruit fly for pathway analysis
- Assessing field attractiveness of lures and sensitivity of trapping grids for *Bactrocera* species and other fruit flies
- Development of a female-specific, tetracycline-suppressed embryonic lethality system for Mexfly

Light Brown Apple Moth Unit

- Develop LBAM SIT technology
- Operate LBAM mass-rearing facility in California
- Conduct tests of SIT release against LBAM in New Zealand and California
- Testing biological control for LBAM in combination with SIT in field cages
- Validate LBAM and European grapevine moth (EGVM) quarantine treatments
- Conduct studies of EGVM phenology and host plants
- Test mating disruption and trapping methods for EGVM

Treatment Quality Assurance Unit

- Support and expansion of Commodity Treatment Information System
- Certify U.S. and international treatment facilities, vessels, and warehouses
- Provide treatment recommendations to PPQ and stakeholders
- Develop and deliver training materials for treatment inspection and databases

- Develop new treatments, including heat treatment of dried plant material, hot water treatment for propagative plant material, and cold treatment for snails
- Review and approve new treatment methods
- Review quarantine treatment research and develop research protocols for testing new treatments
- Continue electronic indexing of agricultural quarantine inspection documents

Director's Office

- Improvement of ACP detection and management methods by sensory evaluation and behavioral modification (Citrus Research Board of California)
- Understanding *Guignardia citricarpa* ascospore production and potential inoculum reduction strategies in Florida (University of Florida)
- Disease modeling via stochastic simulation to test disease control and mitigation strategies to maximize regulatory intervention (University of Cambridge)
- Monoclonal antibody development for *Citrus leprosis virus*, citrus variegated chlorosis, and HLB (USDA-ARS)
- Transmission of *Xyllela 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, UK)
- Monitoring risk of introducing *Phytophthora ramorum* into nurseries and landscapes in southeast USA-Year 4 (Clemson University)
- Rapid detection of *Phytophthora ramorum* in water (Clemson University)

Farm Bill-Funded Projects

- Engineering mature citrus with DiseaseBlock® to achieve immunity against citrus greening (Integrated Plant Genetics)
- Barberry Cultivar Identification and Survey (Michigan Department of Agriculture)

Appendix C. Acronyms and Abbreviations

AAVLD	American Association of Veterinary Laboratory Diagnosticians
ACP	Asian citrus psyllid
AGM	Asian gypsy moth
ALB	Asian longhorned beetle
APHIS	Animal and Plant Health Inspection Service
AQI	Agricultural quarantine inspection
AQI&PT	Agricultural Quarantine Inspection and Port Technology
ARS	Agricultural Research Service
BCU	Biological Control Unit
CANARY	Cellular Analysis and Notification of Antigen Risk and Yield
CAPS	Cooperative Agricultural Pest Survey
CBP	Customs and Border Protection
CBS	Citrus black spot
CDFA	California Department of Food and Agriculture
CFU	Colony-forming unit
CHRP	Citrus Health Response Program
CiLV	*Citrus leprosis virus*
CiLV-C	*Citrus leprosis virus-cytoplasmic*
CPHST	Center for Plant Health Science and Technology
CTIS	Commodity Treatment Information System
DHS	U.S. Department of Homeland Security
DoD	U.S. Department of Defense
EAB	Emerald ash borer
EDP	Emergency and Domestic Programs
EGVM	European grapevine moth
FAS	Foreign Agricultural Service
FY	Fiscal year
GAP	Gradual advance plan
HLB	Huanglongbing (also known as citrus greening)
IFA	Imported fire ant
ISO	International Organization for Standardization
ITP	Identification Technology Program
LBAM	Light brown apple moth
LC/MS	Liquid chromatography/mass spectrometry
Medfly	Mediterranean fruit fly
Mexfly	Mexican fruit fly
NAPPFAST	North Carolina State University/APHIS Plant Pest Forecasting System
NORS-DUC	National Ornamental Research Site at Dominican University of California
NPAG	New Pest Advisory Group
NPDN	National Plant Diagnostics Network
NPPLAP	National Plant Protection Laboratory Accreditation Program
NPRG	New Pest Response Guidelines
PBW	Pink bollworm
PCR	Polymerase chain reaction
PERAL	Plant Epidemiology and Risk Analysis Laboratory
PIS	Plant Inspection Station
PPQ	Plant Protection and Quarantine

PPV	*Plum pox virus*
PRA	Pest risk analysis
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
RPA	Risk and pathway analysis
RRST	Response and Recovery Systems Technology
SDI	Survey, Detection, and Identification
SIT	Sterile insect technique
SOS	Sweet orange scab
TAMUK	Texas A&M University-Kingsville
TQAU	Treatment Quality Assurance Unit
TSA	Tropical soda apple
TWG	Technical working group
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey

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