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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CPHST Web site: www.aphis.usda.gov/plant_health/cphst

Cover Photo: Thermal image of log section during radiofrequency treatment (USDA/APHIS, Ron Mack). This work is described in the project highlight “Development of Radiofrequency as Part of the Dielectric Heating Standard for Solid Wood Packing Material.”
Message from the Executive Director

The Center for Plant Health Science and Technology (CPHST) provides scientific and technical support for the regulatory decisions, policies, and operations of the Animal and Plant Health Inspection Service’s (APHIS) Plant Protection and Quarantine (PPQ) program in order to safeguard U.S. agriculture and natural resources and facilitate safe trade in agricultural products. CPHST ensures that PPQ has the information, tools, and technology to make the most scientifically valid regulatory and policy decisions possible. CPHST also ensures that PPQ’s operations have technically feasible and practical tools for pest exclusion, detection, and management. The 2013 CPHST Accomplishments Report is intended to offer an in-depth look at the status of our programs and the progress we have made toward the Center’s mission and long-term strategic goals.

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

In October 2012, PPQ implemented a new organizational structure with three core functional areas: Policy Management, Field Operations, and Science & Technology. CPHST is the main component of S&T, which also includes the National Clean Plant Network program. With the concurrent implementation of PPQ’s modernization efforts and a new CPHST leadership structure, CPHST commissioned APHIS Policy and Program Development to conduct a review of the CPHST network of laboratories during 2014. This review is designed to assess how well the organization is positioned to support PPQ’s scientific and technical needs, now and in the future.

CPHST had several significant accomplishments in 2013. A few highlights described in this report include:

**Management for Asian citrus psyllid and diagnostics for citrus huanglongbing.**

The CPHST Mission Laboratory has made significant progress in rearing and release methods for the biological control agent, *Tamarixia radiata*. This parasitoid wasp is being used to augment management methods for the Asian citrus psyllid, the vector of the devastating citrus disease, *huanglongbing* (HLB, citrus greening). Biological control is a tool to reduce psyllid populations, particularly for unmanaged citrus in the urban environment. In 2013, using greenhouse mass production methods, over 869,000 parasitoids were reared and released throughout the Lower Rio Grande Valley in Texas. Psyllid parasitism rates of 55-70% have been observed in release sites. CPHST also provided Mexico with 50,000 parasitoids per month to release in the state of Tamaulipas across the border from the Lower Rio Grande Valley. CPHST scientists are transferring the rearing methods to state and industry cooperators in citrus producing states to establish new rearing programs.

In a novel program, CPHST has partnered with local Master Gardeners and homeowners to rear parasitoids in field cages placed around residential trees to increase release numbers and protect trees in urban areas. Average production per cage is over 11,000 parasitoids. This program has been an important outreach tool for the local community in south Texas.

To improve the survey and early detection of HLB, the CPHST Beltsville Lab made significant enhancements in DNA diagnostic methods for this pathogen. The lab developed a new combination assay to detect all three species of Liberibacter bacteria in one assay. In addition, CPHST has reduced Asian citrus psyllid extraction and assay costs by 80%. Another assay improvement reduced the number of inconclusive diagnostic assays for federal confirmation testing by up to 80%. 


Safe international movement of wood products

In the area of phytosanitary treatment development, the CPHST Otis Laboratory has conducted research to demonstrate the efficacy of dielectric heating (microwave and radio frequencies) for use against wood pests and pathogens. CPHST scientists provided technical information to the International Plant Protection Convention, which resulted in inclusion of treatments using dielectric heating in the international standard (ISPM-15) for treatment of wood packing material to prevent the spread of wood pests. This also provides an additional alternative to methyl bromide fumigation. In addition, the Otis Lab provided technical data to support an increase in temperature for the heat treatment standard for the domestic movement of wood, which permitted safe movement of regulated wood and wood products.

Another significant project related to international movement of wood products was conducted by the Plant Epidemiology and Risk Analysis Laboratory to support the Department of Defense (DoD). The DoD has been required to conduct phytosanitary treatments of wood ammunition boxes before entry into the European Union since 2007. However, there were 1200 metric tons of untreated boxes in inventory from before 2007. Treatment of these boxes would be very costly and a safety risk, since it would require unloading and loading of explosive materials. PERAL conducted a pathway analysis to demonstrate that there was negligible risk from these boxes, to support the DoD’s request for an exemption from the treatment requirement. Once finalized, this exemption is expected to save the DoD millions of dollars in treatment costs, in addition to minimizing delays of critical ammunition shipments and avoiding treatment-related environmental impacts.

Partnership on new plant pest detection systems with Department of Homeland Security

CPHST initiated a new partnership with the Department of Homeland Security Science and Technology (DHS S&T) directorate to collaborate on research and development of novel plant pest detection systems that will address the needs and requirements of DHS and PPQ stakeholders that conduct inspections. In order to improve the efficacy and throughput of inspections, PPQ and DHS S&T cooperated to gather the requirements and scan the scientific horizon for new technologies. This project resulted in a recommendation to explore four technology areas: volatile detection, acoustic detection, near-infrared seed sorters, and hyperspectral imaging. This collaboration is continuing and DHS is funding additional projects to further investigate these technologies.

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

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

Office of the Executive Director, Raleigh, NC

Executive Director: Philip Berger
Associate Directors: Russ Bulluck, Michael Hennessey, Charla Hollingsworth, and Ron Sequeira

CPHST is the major component of PPQ’s Science and Technology (S&T) core functional area, which also includes the National Clean Plant Network (NCPN) program. The S&T Office of the Executive Director provides administrative support to CPHST laboratories, coordinates with PPQ programs on project needs and priorities, and provides cross-laboratory program coordination. In addition, this office provides scientific input on initiatives within PPQ and represents PPQ on scientific issues and collaborations with other Federal agencies and partners.

Recent Accomplishments

• Provided scientific support for the Citrus Health Response Program and the newly formed Huanglongbing Multi Agency Coordination Group (MAC). The MAC was formally chartered by the Secretary of Agriculture following stakeholder meetings in the summer of 2013. The group is charged with coordinating citrus research efforts within USDA, industry, state and local governments and implementing field ready solutions to the HLB problem over the next two years. S&T will play a vital role in administering the cooperative agreements resulting from the $21 million portfolio.
• Coordinated and provided administrative support for citrus health-related cooperative projects as well as internal projects.
• Established a working group to promote and coordinate scientific communications between S&T laboratories. The group sponsors and hosts monthly webinars highlighting research activities of the scientists in the various S&T laboratories. Additionally, the group will facilitate webinars delivered by external speakers.
• Established a cross-functional working group (CFWG) to address seed health issues. This effort was the precursor for the first Seed Summit held during the summer of 2014. Stakeholders met to discuss regulatory and non-regulatory options for mitigating open pathways for seed transmitted diseases.
• Led S&T efforts on emergency response to the Cucumber green mottle mosaic virus incursion in California. This included leading a technical working group to address survey, detection and mitigation options for the affected areas.
• Provided support for the National Ornamentals Research Site at Dominican University of California (NORS-DUC). NORS-DUC focuses on systems approaches for nursery production and on developing science-based best management practices and risk mitigation to exclude, contain, and control the regulated pest Phytophthora ramorum from the nursery production chain. The staff has worked with various nurseries throughout California to provide training on the use of steaming equipment for soil mitigation of P. ramorum.
• Assisted in development and deployment of a new P. ramorum Federal Order with additional survey and sampling requirements. CPHST scientists are part of the team that conducts the on-site inspections and assessments.
• Coordinated scientific input for new potato cyst nematode guidelines and participated in a program review.
• Coordinated New Pest Response Guideline document drafts with PPQ headquarters staff.
• Developed and led Technical Working Groups as needed. Provided TWG reports and analyses for bilateral discussions and program reviews.
• Represented PPQ on interagency groups focused on biosurveillance and emergency response.
• Provide leadership and appropriate scientific information to PPQ programs and Plant Health Emergency Response for numerous plant health emergencies: coconut rhinoceros beetle, boxwood bight, Plum pox virus, bleeding canker of horse chestnut, laurel wilt, Megasotpha cribraria, Phytophthora ramorum, chrysanthemum white rust, gladiolus rust, potato cyst nematode, Karnal Bunt, Anguina funestra, and others.
• Successfully piloted radio-frequency identification tracking technology at post entry quarantine sites in Arizona and Oregon. This technology was evaluated as a replacement for paper tracking of plants in post entry quarantine.
• Developed and delivered methyl bromide fumigation schedules for both the pathogen and beetle involved in thousand cankers disease of walnut wood. This treatment will be used extensively to prevent interstate spread.
• Data supporting generic fumigation, cold, and hot water treatments were obtained in vitro for ten species of exotic tephritid fruit flies. Because species were tested side-by-side, we know which species were most tolerant to the treatments. This information will be used to support emergency domestic use of these treatments or use in import trade.
• Obtained fumigation data validating a more effective treatment for Chilean flat mite on figs through collaboration with the Agricultural Research Service (ARS).
CPHST AQI Laboratory, Miami, FL and Raleigh, NC

Laboratory Director: Woodward Bailey

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

Recent Accomplishments
Treatment Schedule Development and Support
- Developed cold treatment schedule for Bactrocera tryoni and Ceratitis capitata on Australian apples, pears, and grapes.
- Developed literature review on the cold tolerance of Bactrocera cucurbitae and B. dorsalis.
- Developed research protocol for cold treatment of fruit flies in Hawaiian citrus.
- Updated research guidelines to support fruit fly cold treatment development.
- With Spanish researchers, developed research protocol for cold treatment of Ceratitis capitata in Spanish clementines.
- Developed cold treatment for Ceratitis capitata in Barhi dates.
- Developed treatment schedule and laboratory protocol for hot water treatment and inspection for Pratylenchus-infested Malus rootstock.
- With Dow AgroSciences and Royal Pest Solutions, developed research plans to validate outdoor breakbulk log sulfuryl fluoride fumigations; developed entry for log exports to China for PExD.

Technical Support for Field Operations and Preclearance Programs
- Certified and approved equipment, facilities, conveyances and officer reports for cold treatment: certified 84,270 refrigerated containers; approved 3 and (re)certified 27 vessels; approved 2 warehouses; approved 2 equipment applications; approved 5316 officer reports.
- Provided cold treatment analysis for the Southern Florida Pilot Study to bring cold treated grapes and blueberries in from Peru and Uruguay.
- Provided on-site cold treatment program training to Field Operations officers and supervisors.
- Reviewed real-time monitoring proposals for in-transit cold treatment.
- Approved facility plans for and certified 1 domestic irradiation facility.
- Approved irradiation packaging and process configurations for Preclearance and Field Operations.
- Conducted a stakeholder-initiated project to evaluate the use of predictive modeling to replace current irradiation process configuration dose mapping requirements.
- Provided update for NAP fumigation chamber certification chapter in PPQ Treatment Manual.
- Approved and certified 4 NAP fumigation chambers.
- Approved fumigation packaging for Preclearance and Field Operations.
- Compiled extensive yearly MB usage report for 2012, including data on commodity, country of origin, reporting station, schedule, contractor, and usage.
- Completed protocol for the analysis of fruit fly patch lure and solid lure for ammonium acetate content and release rate.

Analytical Chemistry Support
- Analyzed samples for PPQ Programs.
  - Fruit Fly Program (78)
  - AQI Program (42)
  - Other CPHST activities (14)
- Assisted DHS to develop a statistical model generated from isotope analysis of elemental signatures of mangos; comparison of three authentic growing regions of California, Florida and Puerto Rico provided distinct data clusters.

Imported Fire Ant Program
- Notice in Federal Register of two treatments to support the IFA Quarantine and the availability of a new emergency action for the program; bifenthrin for nursery stock immersion/dip and bifenthrin for grass sod.
- Completed revision of APHIS Program Aid 1904 with new treatments and distributed to SPHDs and SPROs in late 2013.
- Continue coordination of IFA-phorid fly releases and began reduction of program in 2013; 16 releases of flies in FY13 (1 or more species per release) in multiple states.

Analysis of Routine Environmental Monitoring Samples
- Contracted with Agriculture Marketing Service for chemistry analysis:
  - Boll Weevil program (1 sample)
  - Asian Long Horned Beetle program (282 samples)
  - Grasshopper Mormon Cricket program (29 samples)
Commodity Treatment Information System (CTIS) Management
- Provided webinar Preclearance Officer training for IRADS.
- Provided webinar Officer and Supervisors training for 429 Fumigation Database.
- Provided on-site officer and supervisor training for 556 Cold Treatment Database.
- Provided ad-hoc analyses to Policy Management, Field Operations, and stakeholders.
- Provided support for CTIS migration to National Information Technology Center.

**Recent Accomplishments**

- Evaluated CANARY sample preparation methods for *Phytophthora* spp.
- Completed Phase 1 of the Online Identification Tools for *Phytophthora*: Lucid Key, Tabular Key and Sequencing Analysis.
- Evaluated of alternatives to *Phytophthora ramorum* ELISA testing.
- Completed Plum pox virus, huanglongbing, and *Phytophthora ramorum* proficiency test panel development for NPPLAP.
- Developed molecular diagnostics methods for the identification of Graminicolous Downy Mildews using conventional PCR and DNA sequence analysis.
- Developed a multiplex one-step reverse transcription (RT) conventional PCR for the detection of *Citrus leprosis* virus-cytoplasmic type-2 (CiLV-C2).
- Developed a multiplex one-step reverse transcription quantitative PCR targeting the coat protein gene for the detection of *Citrus leprosis* virus-cytoplasmic type-2 (CiLV-C2).
- Analyzed completed genome assembly of *Citrus leprosis* virus-nuclear types to reveal a close association with *Orchid fleck virus*.
- Developed a multiplex one-step RT-PCR for the detection of Nepoviruses in Subgroup C.
- Developed a multiplex RT-qPCR for the detection of sweet potato infecting Potyviruses.
- Evaluated and developed a new protocol for Asian citrus psyllid (ACP) pooling for DNA extraction.
- Evaluated primers for *Candidatus Liberibacter asiaticus* 16S rDNA with or without the G Insertion in the species for its significance for disease detection.
- Developed molecular diagnostics methods for universal phytoplasma detection.
- Developed a duplex conventional PCR for the detection of *Xylella fastidiosa* at the species level and differentiation of strains.
- Adapted two duplex real-time PCR assays for citrus variegated chlorosis (CVC) screening for use on the ABI 7500 PCR System.
- Developed and validated a process of direct sequencing duplex conventional PCR products to speed up the U.S. federal confirmatory test for *X. fastidiosa* CVC strains.
- Incorporated two duplex real-time PCR assays into one triplex for CVC screening on Cepheid SmartCycler platform.
- Developed molecular diagnostics methods for the sweet orange scab fungal pathogen *Elsinoë australis*. 

**CPHST Beltsville Laboratory, Beltsville, MD**

**Laboratory Director: Mark Nakhla**

The CPHST Beltsville Laboratory has two main missions: 1) To develop, adapt, 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. 2) To provide the official federal identification of plant pathogens of regulatory significance, which is the basis for federal regulatory actions and policies.

The laboratory programs utilize cutting-edge technologies from the fields of plant pathology, molecular biology, human and animal clinical diagnostics, and bio-detection to develop, adapt, and improve methods for the accurate and rapid diagnosis of plant pathogens. The laboratory validates plant pathogen diagnostic methods prior to stakeholder release to assure their performance and fitness for use in regulatory programs. Beltsville Lab strives to achieve timely transfer of field deployable diagnostic tools for PPQ emergency response and eradication programs. Beltsville laboratory provides operational diagnostics of high-consequence and select agent plant pathogens requiring Federal confirmation.

The Beltsville Lab is a key component of the PPQ National Plant Pathogen Laboratory Accreditation Program (NPPLAP). Beltsville is responsible for proficiency test panel development, delivery, and first-level evaluation for scientists who perform diagnostics on behalf of PPQ using CPHST-validated methods. The laboratory is committed to quality in biochemical and molecular diagnostics and is proficiency tested in the operation of diagnostic methods. Beltsville staff conduct outreach to the plant pathology diagnostic community by providing technical support—protocols, hands-on laboratory training, and troubleshooting for PPQ-validated diagnostics—to scientists within the National Plant Diagnostic Network (NPDN), PPQ port and regional identifiers, and State departments of agriculture. Beltsville scientists also contribute their expertise in detecting regulatory plant pathogens by serving as members of scientific groups and committees. The CPHST Beltsville lab is internationally recognized and has developed a strong international collaborative network through participation in technical working groups, collaborative projects, and direct scientist interactions.
• Developed molecular tools for the detection of the citrus black spot fungal pathogen *Gaignardia citricarpa* by conventional multiplex PCR.

• Developed molecular diagnostic methods for the late wilt of maize fungal pathogen *Harpophora maydis*.

• Provided tours and presentations to international visitors from 12 different countries to assist with international technical regulatory capacity building.

• Completed and released 42 new of revised work instruction documents. These covered a diverse range of diagnostic methods, training instructions, nucleic acid isolation protocols, and quality management processes.

• Provided on-site training on ten different plant pathogens to scientists and diagnosticians from PPQ, NPDN and state universities. In addition, two staff provided off site training on general plant pathology, plant permitting, and Real Time PCR.

• In FY 2013, completed regulatory diagnoses for a total of 17 different plant pathogenic organisms. A total of 1237 individual samples were tested and determinations made and reported. Of these, 516 of the samples tested negative, 442 were positive, and 135 were inconclusive for the pathogen of concern. In addition, 136 samples were rejected for quality issues or did not meet regulatory policy. Twelve samples were part of a collaborative project with ARS.

**CPHST Fort Collins Laboratory, Fort Collins, CO**

**Laboratory Director: Richard Zink**

Work at CPHST’s Fort Collins Laboratory focuses on five critical areas of methods development for APHIS: pest identification technologies, pest survey protocols and guidelines, risk mapping, AQI waste disposal and decontamination, and biological control. The laboratory provides extensive scientific support for the Cooperative Agricultural Pest Survey (CAPS) program. The laboratory transfers methodologies and tools to field operations to ensure efficient and effective survey, detection, identification, emergency response, and eradication efforts. The Fort Collins Lab also develops electronic, matrix-based identification resources to help support rapid, consistent, and accurate identification and nomenclature of pest species.

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

**Recent Accomplishments**

**CAPS Support**

• Released the “Palm Commodity-Based Survey Reference.” The Introduction document contains information on survey planning, trapping instructions, and sample submission. The Reference includes datasheets for 12 pests.

• Released pest datasheets for one nematode, eight insects, and nine plant pathogens. Datasheets were also revised for four plant pathogens, sixteen insects, two nematodes, and one mollusk.

• Developed the 2014 Analytical Hierarchy Process (AHP) Prioritized Pest List using a new process: 1) Pre-assessment, 2) existing AHP model, and 3) Post-assessment. The Pre-assessment questionnaire is used to assess new CAPS pest suggestions before the pests are run through the AHP model. The Post-assessment questionnaire evaluates the survey and identification/diagnostics methods of pests. For the 2014 list, six pests did not pass the Post-assessment and were removed from the final AHP list. These pests were moved to a research list which is used to prioritize funding for methods development.

**Field releases of the Russian knapweed gall midge**

• In 2013, we continued our greenhouse-based rearing program for the gall midge, *Jaapiella ivannikovi*, a classical biological control agent of the invasive weed Russian knapweed, *Rhaponticum repens*. We provided 942 galls for release at 18 sites in seven states (California, Colorado, Idaho, Oregon, Utah, Washington, and Wyoming), with about 52 galls released, on average, at each site. Two releases were used to augment sites initiated in 2011 or 2012, while 16 occurred at new sites. To date, the FCL rearing program has provided *Jaapiella ivannikovi* for field release at 72 sites in eight western states (the seven mentioned above and New Mexico). At least 25% of these sites have established midge populations.

**Identification Technology Program (ITP) produces Mobile Apps for Field Screening Support**

• During 2013, ITP initiated projects for development of mobile web apps and Android and iOS native apps. In partnership with the University of Florida’s IFAS Information Technology, Texas A&M University, and University of California, ITP announced the release of NPDN Citrus Pests and NPDN Citrus Diseases as mobile web apps and native Android and iOS apps. These screening aid apps, aimed primarily at extension agents and inspectors, include fact sheets, illustrations, glossaries, and images. The partners also initiated work on the ProCitrus app, a symptom based app to be released in 2014 to support industry with detecting and screening pests and diseases of citrus. In collaboration with the University of Queensland’s QAFAI Biological Information Technology team (QBIT), ITP selected 10
desktop Lucid keys from ITP’s existing tool arsenal to convert to Lucid Mobile. Lucid Mobile takes full advantage of all the conventions and functionalities on mobile devices, while supporting the features of desktop Lucid keys.

Identification Technology Program Releases

- **Diabrotica ID:** Identification of North and Central American Diabrotica Beetles is designed to allow identification of *Diabrotica* species for users lacking an expertise in taxonomy of the genus, one of the most speciose and economically important leaf beetle genera in the New World <http://idtools.org/id/beetles/diabrotica/>.

- **Flat Mites of the World Edition 2** includes a number of taxonomic revisions and new species that were discovered as a result of the research performed as part of the development of the first edition <http://idtools.org/id/mites/flattmites/>.

- **Hispines of the World,** developed for non-specialist users, this tool aims to support the identification of hispine beetles, many of which are major pests of economic importance <http://idtools.org/id/beetles/hispines/>.

- **Microlepidoptera on Solanaceae** focuses on the two largest taxonomic groups of moths that feed on solanaceous crops and is the first publication that brings information about these species together <http://idtools.org/id/lcps/micro/>.

Implement a Web-based Application for Sharing Geospatial Data, Using Historic Barberry Eradication Records as a Model

- The geospatial database to support the Black Stem Rust/Barberry program has been further developed and now includes over 13,000 records from eight states (ID, MT, WA, OR, ND, SD, WY and WI). To visualize the data outside the APHIS firewall, the project has partnered with geospatial resources at Kansas State University, and final data serving is planned for summer of 2014. Overall goals of the project are to: (1) create a permanent electronic archive of historical survey data for visualization and query; (2) use the archive to develop tools for early warning of any new races of the rust pathogen appearing in wheat-growing regions; and (3) assist in current barberry survey efforts by directing staff to where the plant existed previously.

Interagency Cooperation to Accomplish Predictive Modeling Goals

- The Fort Collins Lab has partnered with sister agencies in the local area to build capacity and technology sharing for responding to agency needs in the realms of geospatial analysis, pest forecasting, and predictive modeling. Collaborative examples include working with the U.S. Forest Service (USFS) Forest Health Technology Enterprise Team for building the European gypsy moth national risk assessment model, and working with U.S. Geological Survey (USGS) Fort Collins Science Center to use their software systems for statistical modeling. These collaborations have yielded mutually beneficial products to both agencies and led to increased technology sharing and modeling advancement.

Molecular Identification of pests at ports: The RAMBO project

- The Rapid Automated Molecular Barcode Observation (RAMBO) project evaluated a molecular diagnostic instrument for possible use to identify agricultural pests.

**CPHST Mission Laboratory, Edinburg, TX**

**Laboratory Director:** Dr. Matthew Ciomperlik

The Mission Laboratory supports PPQ’s programs by developing detection and management methods, mitigation strategies, and molecular diagnostic tools for invertebrate pests and plant pathogens. Our scientists work with international groups, federal and state agencies, industry, academia, and growers to identify, develop, and deliver effective scientific methods that help to reduce the introduction of unwanted organisms and improve upon the management and eradication strategies for established plant pests. In addition to these core functions, we provide expertise in epidemiology of plant diseases, remote sensing/geographic information systems, biological control, area-wide pest management, and sterile insect technology (SIT) program support.

Our scientists possess a wide range of skill sets that include current and informative trends in molecular biology. This scientific expertise provides the Mission Lab, and PPQ, the capacity to develop effective molecular diagnostic tools used in confirmatory identifications, determine high-risk pathways of invasive species, and screen genomes for diagnostic markers.

One example of a novel application of molecular diagnostic tools incorporates DNA and RNA extraction methods to identify individual flat mites (*Brevipalpus* spp.) to species and detect *Citrus leprosis virus* (CiLV) in a single mite. The analysis couples DNA diagnostic methods developed at the CPHST Mission Laboratory and RNA based markers for CiLV developed at the CPHST Beltsville Laboratory. These in-house techniques and skills are supporting PPQ programs through the development of identification tools and integrative projects to understand pest and vector distributions, dispersal, introduction pathways, and behaviors.

**Recent Accomplishments**

**Molecular Diagnostics**

- Developed a method to co-isolate genetic material from both *Citrus leprosis virus* and its flat mite vectors (*Brevipalpus* sp.). The protocol is the first available method for testing CiLV from a single mite and can be used to
Documented genetic diversity of oriental fruit flies (Bactrocera dorsalis) trapped in Hawaii and California to test an introduction pathway for the exotic pest. This work shows that Hawaii is not a likely source for half of the flies trapped in California from 2008 to 2012 and supports APHIS fruit fly exclusion efforts that target multiple introduction pathways.

Completed molecular studies of the West Indian fruit fly (Anastrepha obliqua) to distinguish geographic populations and confirm taxonomic status of the fly as a single species. The differences observed between populations support pathway analysis of this offshore pest.

Completed development of the Medfly Molecular Tool (MMT), a DNA-based resource for pathway analysis of the Mediterranean fruit fly (Ceratitis capitata). The MMT synthesizes multiple molecular technologies into one procedure to provide increased confidence in source estimation of this invasive pest. The MMT has confirmed suspected introduction pathways into the United States and has the capacity to improve resolution in future pathway applications.

Finalized development of a real-time PCR protocol to screen gypsy moths (Lymantria dispar) trapped in the United States for evidence of Asian gypsy moth (AGM) DNA. This new method efficiently genotypes moths and is compatible with ongoing AGM detection and exclusion activities.

Developed a real-time PCR assay to diagnose the Khapra beetle (Trogoderma granarium). This new test provides a timely and reliable method to confirm the identity of suspect Khapra beetles collected in stored grains to support early detection of this highly invasive pest.

Mexican Fruit Fly Program Support

Delivered a monitoring tool for the effectiveness of Mexfly SIT releases to the PPQ program. The tool is used to determine optimum sterile release rates for quality assurance, and helps in decision making for initiation of quarantines when mated female Mexfly are captured. The tool was successfully used to avoid the initiation of 2 separate quarantines during the 2012/13 citrus season.

Developed shipping, egg bubbling, and handling procedures for Guatemalan-produced Mexfly eggs that produced large number of high quality flies for use in sterile insect releases. This is the first time Mexfly eggs shipped from outside the United States have been used for domestic fruit fly control programs.

Conducted calibration and quality control testing of aerial and truck mounted release machines to ensure quality and effective release of Mexfly. The resulting release graphs provided values of flies released based on vehicle speed, release swath, and adjustable potentiometer settings. Results were used to update standard operating procedures for aerial release and provide the basis for new release procedures for ground releases.

Determined that Morganella sp., Providencia rettgeri and Providencia alcalfaciens bacterial isolates are pathogenic to Mexfly larvae and can cause rearing failures due to their effect on decreasing insect viability.

Determined that several ready to use surface disinfectants are effective in controlling Morganella sp., Providencia rettgeri and Providencia alcalfaciens. Results also indicated that two antimicrobials will detrimentally affect the viability of Mexfly larvae.

Citrus Health Response Program

Over 650,000 Tamarixia radiata were mass reared and released for control of ACP, at over 380 sites in the Lower Rio Grande Valley (LRGV) of Texas, with emphasis on releases in quarantined areas. Parasitoid recoveries were confirmed at 41 locations, 19 of those sites are within quarantined areas.

A collaborative project was initiated with APHIS-IS and SENASICA of Mexico to begin releases of parasitoids for ACP in Tamaulipas along the Texas/Mexico border. Provided APHIS-IS with over 159,000 biological control agents of ACP which were released at 45 sites in Mexico along the Texas border.

Updated the LRGV commercial citrus orchard survey using 2013 aerial imagery and ground truth survey of all citrus orchards. This information will be used by APHIS PPQ and the Texas citrus industry to help manage the Asian citrus psyllid area-wide management program, target high risk areas for citrus greening disease (HLB) scouting, and model the spread of HLB in the region.

The CPHST Mission Lab is working with PPQ Field Operations Citrus Survey and the Texas A&M Kingsville Citrus Center diagnostic lab to develop the data necessary to conduct a hot-spot cluster analysis to model the proximity of suspected HLB-positive psyllid samples to HLB-positive and -suspect plant tissue samples. Preliminary analysis of the data has helped in targeting specific areas for collecting plant tissue samples.

Documented the distribution of Candidatus Liberibacter asiaticus within citrus trees in collaboration with the Texas A&M Kingsville Citrus Center. This work demonstrates that detection from root tissue is more precise than leaf tissue. Improved efficiency will aid ongoing efforts to limit the spread of HLB disease.

Completed testing organically certified products for disinfecting citrus fruit affected by sweet orange scab (SOS). This work identified three products with equivalent effectiveness to current accepted protocol.

Developed methods for isolation of SOS using two new culture media, Rose Bengal and Malt Yeast Agar, both amended with Dodine. The new methods increased frequencies of recovery of Elsinoë australis with reduction in contaminant saprophytic fungi.

Provided data to determine approach rate of Brevipalpus sp. mites, vectors of citrus leprosis virus to DHS-CBP and CPHST PERAL for risk analysis.
CPHST Otis Laboratory, Otis Air National Guard Base, Buzzards Bay, MA

Laboratory Director: Vic Mastro

The CPHST Otis Laboratory’s mission is to identify, develop, and transfer technology for survey, exclusion, control, and risk assessment on behalf of APHIS and its cooperators. The lab serves a wide variety of PPQ programs, including exotic pest detection, agricultural quarantine and inspection (AQI), Asian longhorned beetle (ALB), European grapevine moth (EGVM), Asian gypsy moth (AGM), emerald ash borer (EAB), and Asian citrus psyllid (ACP), among others. Otis personnel identify high-risk exotic pests and develop methods to exclude, detect, delimit, and mitigate effects of those pests.

The Otis Lab was established on Cape Cod in the 1960’s as a gypsy moth laboratory. Though the lab’s scope has since broadened, it continues to support the PPQ’s gypsy moth programs by developing molecular methods to distinguish among subspecies of the pest and helping ensure the quality of lures and traps.

To fulfill the lab’s mission, Otis personnel maintain cooperative relationships with other Federal agencies, such as ARS, USDA’s Forest Service, and DHS’s Customs and Border Protection, as well as state departments of agriculture, universities, and private industry. Additional cooperative arrangements extend to government agencies, international organizations and universities in a number of foreign locations, including Austria, Canada, China, Japan, Korea, New Zealand, Russia, and South America. The work includes developing methods to monitor and exclude exotic pests from North America, predicting the invasiveness of organisms by assessing damage on expatriate North American plants in foreign locations, developing and evaluating attractants and traps, developing phytosanitary treatments for commodities and packaging materials, and evaluating control techniques for targeted exotic pests.

Recent Accomplishments

Pest Detection

- Continued to identify and field-test attractants for wood boring beetles through a project funded by the Farm Bill. To date, attractants for over 100 species of longhorned beetles have been identified, and tests to confirm their activity are being run in numerous locations around the world. Specific information on attractants for cerambycids and exotic buprestids was given to the CAPS program for incorporation in the CAPS wood borer survey.
- Prepared and distributed approximately 100,000 pheromone dispensers to participants in CAPS and national surveys.
- Projects were initiated, though Farm Bill funding, to develop attractants and trapping methods for the polyphagous shot hole borer and fruit-piercing moth.
- Field-tested new multi-species and multi-use traps in domestic and foreign locations on a number of surrogate and CAPS target pests. Continued evaluations of new insect glues that reduce mess for trappers and ease identification of captured insects, and made initial recommendations for their use to the CAPS program.
- Completed third year of pilot testing a risk-based model for emerald ash borer detection trapping with positive results. Provided consultation on a similar project on European gypsy moth.

Diagnostic Tools

- Developed additional markers for gypsy moth mitochondrial DNA, allowing 5 regions to be sequenced. This substantially increases the amount of molecular information available for phylogenetic analyses of gypsy moths based on the mitochondrial genome.
- Discovered ~4000 single nucleotide polymorphisms (SNP’s) among three broad populations of gypsy moth (North American, Chinese, and Russian). Allele frequencies of those SNPs are being examined for differences that are fixed, or nearly fixed, between different Asian and North American populations.
- Used DNA sequencing to identify immature stages of Asian defoliators that were intercepted on ships and containers. Also developed a potential method of distinguishing among gypsy moth, rosy moth, and Casuarina tussock moth (L. xylina) based restriction fragment length polymorphisms (which, unlike sequencing, does not involve mail-out).
- Standardized our techniques for sequencing mitochondrial DNA of Asian longhorned beetles (ALB) and successfully “barcoded” specimens from Toronto, Canada, Farmingdale, NY, and Michigan (one intercepted

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Asian Longhorned Beetle

- Began work to develop a common database for ALB DNA sequences, which will facilitate comparisons among all ALB populations for which molecular data are available.
- In an ongoing port monitoring project, immature wood borers intercepted in wood packing material from six ports were received to rear to the adult stage. Adults are reared from larvae and are being identified and used to generate a DNA barcode for the intercepted species. DNA analysis can then be used to identify future interceptions of larval wood borers, which usually cannot be identified to species by standard means. Species identifications are being tied with interception and wood treatment data to uncover high-risk patterns of non-compliance by wood treatment facilities and counties.

Emerald Ash Borer

- Completed life-table analysis of EAB and its natural enemies in its native range (China and Russia) on ash trees native to Asia and North America.
- Initiated study of EAB parasitoid dispersal along a linear ash corridor in NY.
- Developed the standards for an online database for recording recovery of EAB parasitoids nationwide. Analysis of these data showed that Spathius agrili can overwinter but is not establishing in the northern U.S.
- Worked with cooperators to continue analysis of the impact of EAB parasitoids in NY, MD, and MI.
- Submitted a permit application for field release of Spathius galinae, which was approved by the North American Plant Protection Organization.
- Supported a graduate student research project to compare the efficacy and efficiency of several methods for recovering EAB parasitoids.
- Following multiple years of testing, a recommendation was made to the program that the green multi-funnel trap is a suitable alternative to the purple sticky trap for general detection and monitoring of EAB populations. An additional recommendation is that Z-3-hexenol can be used alone for an attractant, creating savings by eliminating manuka oil from EAB lures. The program is planning to phase in these recommendations during upcoming years.
- Continued tests on newer EAB trap designs and colors in foreign locations, with results indicating that these traps will be useful for monitoring a number of Agrilus species of concern.
- Developed and tested a field cage for propagating EAB parasitoids under natural conditions. This “field insectary” will be used to disperse the parasitoids rapidly into areas newly invaded by EAB and to facilitate their rearing by state agencies.
- Demonstrated color preferences in the landing behavior of the EAB parasitoid Spathius agrili, and made recommendations on the color of traps for monitoring Spathius populations.
- Initiated an evaluation of the depth of EAB pupal chambers across a range of ash log diameters. The resulting data will be used to determine if debarking large (>12”) diameter ash material will make it safe for processing into commercial lumber without the need for additional treatment.
- Developed artificial substrates palatable to adult emerald ash borer and Asian longhorned beetle – a critical first step toward artificial diets for adult beetles, which could greatly streamline laboratory rearing of these species.

Sirex woodwasp

- Assembled a compendium describing the history of work and accomplishments in Sirex methods development by Otis scientists and their cooperators.
- Field and wind tunnel tests were used to evaluate attraction of Sirex noctilio to host volatiles, pheromone,
and visual cues, including the roles ultraviolet light and visual silhouettes of trees as compared to odors.

Invasive Forest Pests
- With Penn State and the Cary Institute for Ecosystem Studies, initiated a multi-year, Farm-Bill-funded modeling study to evaluate combined effects of multiple invasive pests on northeastern U.S. forests. Ecological and economic consequences are considered. Initial species include Asian longhorned beetle, emerald ash borer, beech bark disease, and hemlock wooly adelgid, but additional pests can be added. The resulting model system will assist in developing risk assessments as well as rationales and justifications for invasive species programs.
- Developed new artificial diet and new rearing protocol for *Euwallacea fornicatus* based on boxelder sawdust and avocado sawdust, and established a laboratory colony on artificial diet in the Otis containment facility for research on behavior and chemical ecology.

European Grapevine Moth (EGVM)
- Completed a technical working group report providing direction and methodology for the successful conclusion of the EGVM program.
- Through cooperators in Italy, conducted tests of alternative mating disruption formulations. Delivered additional recommendations on lures that can be used when a mating disruption regimen for EGVM is in place.
- Good progress was made in studies to determine effects of a range of gamma radiation doses on reproductive potential of various ages of male EGVM pupae. Data generated, along with continuing advances in mass rearing, provide a basis for potential SIT and Inherited-SIT programs, which would expand control options for this invasive pest.

Asian Gypsy Moth/Gypsy Moth
- Supported the AGM exclusion program by participating in bilateral or trilateral (with Canada) meetings in Japan, Korea. Conducted cooperative work in AGM source countries to define the range, population pressure, and behavior of AGM adults. These consultations and the technical input have resulted in a strengthened offshore inspection/certification program for AGM.
- During FY 2013, the Otis Lab discontinued a long-standing cooperative project of providing the Forest Service with cadavers of virus-infected gypsy moth caterpillars for the production of Gypchek a species-specific, EPA-registered biopesticide. The Forest Service now contracts a private company for this service, though the Otis Lab will continue providing the private firm with gypsy moth eggs until their rearing effort come fully on line. The lab’s gypsy moth colonies remain a global resource for research and outreach projects.

Phytosanitary Treatments
- Provided technical information on the use of dielectric (radio frequency and microwave) heat, leading to its acceptance as a treatment for wood packaging material by the International Plant Protection Convention (IPPC) in spring 2013. Additional studies will be conducted to facilitate commercial scale-up of this treatment method.
- Continued work with IAEA toward the development of a generic cold treatment for tropical tephritid flies. A generic protocol would simplify issues around treatment fruit and allow trade in fruit from areas known to be infested with these pests.
- Represented PPQ on international panels overseeing phytosanitary standards, including the IPPC Forestry Panel, IPPC Technical Panel for Phytosanitary Treatments, and Phytosanitary Temperature Treatments Expert Group.
- Initiated projects to develop phytosanitary treatments that provide alternatives to methyl bromide for use against khapra beetle, including alternate fumigants and irradiation.
- Completed testing on use of vacuum steam as a phytosanitary treatment for commercial loads of hardwood veneer logs.
- Collaborated with researchers at Penn State to successfully model heating uniformity and depth of penetration for the new ISPM-15 dielectric heating standard. Represented PPQ as a steering committee member and made recommendations to other IPPC technical panels on treatment development for ISPM-15.
- Completed testing and finalized a new spray schedule (T409-b-3) for insecticide use in cargo holds and containers to disinfest for Japanese beetle and other flying insect hitchhikers. This need was in response to losing the EPA registration for our only aerosol product (10% d-phenothrin).
- Provided technical data under an interagency agreement with ARS to support development of commodity treatment against EGVM based on the minimum radiation dose required to prevent development of immature stages to the adult stage.

California Station
- Developed a small-scale rearing system for EGVM capable of producing up to 10,000 larvae per week. This colony was used to test pesticides and develop improved tools for the EGVM area-wide control program; the efficacy of new and existing materials and application rates were both tested. This information was delivered to growers through University of California Cooperative Extension.
- Cooperated with the University of California (UC) and ARS to develop fumigation schedules for table grapes using methyl bromide and other treatment alternatives. To support this work, a custom environmental control room for fumigation work for EGVM on table grapes was designed and installed inside a UC-Davis quarantine lab.
- Completed several projects with UC on EGVM mortality during wine-making procedures. The work showed that there is no risk of spreading EGVM by transporting red
wine must, which allowed this commodity to be deregulated. The project results also demonstrated that regulatory treatment during the press process of 2 bars of pressure is effective, and that the grape crush process can allow significant numbers of larvae and pupae to survive if grape loads come from infested fields. These results highlight the need for increased sanitation at wineries that may be processing infested grapes.

- Supported the EGVM control program by conducting studies of phenology and surveys of alternate host plants in Napa County.
- Organized a working group with the strawberry industry, Monterey and Santa Cruz counties, LBAM Cooperative Program personnel, and UC to conduct a pest risk analysis of LBAM and develop a systems approach for conventional and organic strawberries that would establish streamlined regulatory procedures for interstate and foreign export of strawberries from quarantined areas.
- Worked with UC to test existing and new materials as regulatory treatments of LBAM on nursery stock. Results showed which materials were most effective to control different LBAM life stages and the duration of effective control on field-grown material. Several treatments had extended control up to 3 weeks after treatment.
- Conducted a study on the use of irradiation as a phytosanitary treatment for LBAM using an X-ray source.
- Analyzed the horticultural mineral oils available in California and provided a recommendation to the LBAM program to allow the regulated industry more options for using these oils to control LBAM on nursery stock. This recommendation was adopted by CDFA, and growers now have more choices in their selection of approved oil treatments.
- Analyzed LBAM program data to estimate the spread rate of LBAM in California and prepared a written recommendation regarding a proposal to reduce the size of the quarantine area for new LBAM detections.
- Analyzed LBAM trapping records and scientific literature to provide a technical recommendation for the size of an effective quarantine area around a new LBAM find.
- Tested a new four-component LBAM pheromone lure under California environmental conditions and determined the use of the new lure would significantly improve trap catch over the current two-component lure.
- Initiated new work to mass rear *Tamarixia radiata* in California. This parasitoid is a biological control of the Asian citrus psyllid, a vector for citrus greening.

**Recent Accomplishments**

**Pink Bollworm Section**

- Aided the PBW rearing facility to investigate the costs and benefits of cooking PBW diet fewer times per week in order to more efficiently feed the smaller numbers of PBW needed.
- Provided insect diet shipments to cooperators.
- Conducted tests to measure escape of moths from the Pink Bollworm Rearing Facility and recommended measures to limit those escapes.
- Conducted quality control of red dye and strontium concentrations in PBW Rearing Facility moths.
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- Conducted quality control of red dye and strontium concentrations in PBW Rearing Facility moths.
- Investigated the viability of using apyrene vs. eupyrene sperm in PBW male sperm bundles as a method to determine if moth was irradiated. The sperm bundles

**CPHST Phoenix Laboratory, Phoenix, AZ**

**Laboratory Director: Richard Zink**

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

The Phoenix Lab’s rangeland section works with Federal and State customers to provide technical assistance for the grasshopper and Mormon cricket control programs. This section also develops and implements solutions to program problems and continuously evaluates the technology and tools of the control program. The PBW section supports the PBW eradication program by providing expertise on pheromone mating disruption, custom rearing and mass rearing of insects, SIT mechanisms, insect population monitoring, and insect behavior. This section works closely with the PBW rearing facility, CPHST Albany facility, Arizona Cotton Research and Protection Council, ARS’ Arid Lands Agricultural Research Center, University of Arizona’s Entomology Department, local cotton growers, International PBW Eradication Program, and State and regional PPQ offices.
were too variable and were not a reliable means to separate mass-reared, sterilized PBW from non-sterilized PBW.

- Set out traps in areas of wild cotton and captured species similar to PBW that were also attracted to the PBW lure or trap. Conducted genetic analysis to positively identify PBW from other, easily confused species and added the identification characteristics to field keys to preserve and share that knowledge and avoid misidentification of pest species.
- Used field trial to compare new “no-mess” Delta trap to standard sticky trap.
- Compared current formalin egg treatment to Oxiver Five 16 and CHG in small scale and in larger scale experiments. Oxiver Five 16 performed very well and will be tested in full scale rearing conditions in 2014.
- Elucidated the role of wild cotton as a poor host for PBW.
- Prepared for small scale releases to respond to trap catches and post-eradication needs by applying for certificate of airworthiness.

Rangeland Section

- Developed a Rangeland Grasshopper Strategic Plan, distributed to relevant parties, and interpreted responses to a questionnaire to help guide CPHST research goals as well as set policy and operational guidelines for the near future.
- Review and updated Biological Assessment for the APHIS Rangeland Grasshopper and Mormon Cricket Suppression Program for mitigation of non-target effects of rangeland pest suppression.
- Participated in APHIS Tribal Consultation Training.
- Supported Utah State University to screen for grasshopper and Mormon cricket pathogens discovered in soil samples collected across the seventeen western states by PPQ domestic personnel while conducting surveys.
- Met with many industry representatives to present results and discuss upcoming field projects.
- Maintained contacts with State Plant Health Directors to locate suitable grasshopper populations for field trials.
- Worked with Program Managers to conduct CPHST trials along with ongoing rangeland grasshopper suppression efforts.
- Maintained contacts with ARS to conduct relevant research at remote sites and plan future research goals.
- Provided resources and support for review of grasshopper IPM Manual for Western Region.
- Worked through PPQ Nebraska, U.S. Fish and Wildlife Service, and Nebraska Game and Parks to solve problems associated with threatened and endangered species (blowout penstemon and American burying beetle) prior to Rynaxypyr operational scale treatment applications in Nebraska.
- Met with PPQ Policy and Operations staff to discuss the Rangeland Grasshopper Strategic Plan, Biological Assessment, need to rewrite the Rangeland Environmental Impact Statement, and the need for additional information before using Rynaxypyr for rangeland pest control.
- Presented research summaries to the National Grasshopper Management Board Annual Meeting.

CPHST Plant Epidemiology and Risk Analysis Laboratory

Laboratory Director: Ron Sequeira

The CPHST Plant Epidemiology and Risk Analysis Laboratory (PERAL) is PPQ’s primary unit producing pest risk analyses (PRAs). PRAs are essential for safeguarding American agriculture and environment from harmful exotic plant pests in both managed and unmanaged ecosystems. More specifically, PRAs help PPQ identify and assess new pest threats, design risk-based regulations for import and domestic pest management programs, monitor the effectiveness of existing programs, and optimize available resources.

The diverse staff of PERAL scientists and professionals provides essential scientific support to risk-based policymaking across a broad range of phytosanitary issues. PERAL staff members apply evidence-based approaches and a variety of scientific methods to analyze import-, export-, and operational issues, in addition to providing technical support documents required by PPQ regarding pests, commodities, and pathways. PERAL products include spatial analyses that indicate existing or potential pest distribution ranges domestically or internationally, or that predict geographic ranges and epidemiological characteristics of invasive species.

PERAL has established itself as a global leader in terms of innovation, productivity, and quality management in the area of plant quarantine issues. PERAL is currently the world’s only plant health risk analysis unit certified under the International Standards Organization (ISO). Through its Risk Analysis Mentoring Program (RAMP) for visiting scientists, PERAL contributes significantly to the promotion of international dialogue and increased capacity for science-based management of phytosanitary issues. In addition, PERAL provides PRA workshops covering concepts, methods, and resources associated with pest risk analysis. PERAL also promotes regional and international harmonization of plant health regulations by providing scientific support to PPQ in the North American Plant Protection Organization (NAPPO) and the International Plant Protection Convention (IPPC).

Recent Accomplishments

- PERAL completed twenty-two Q-56 and six Q-37 PRAs in 2013. The import requests addressed by these PRAs comprised 44 country-commodity combinations, and the
average time required for completion for each PRA was 205 days. In addition, PERAL revised sixteen Q-56 PRAs that were completed in previous years but have not yet completed rulemaking.

- PERAL screened approximately 500 plants species for their threat potential to the United States. Candidates for listing as Federal Noxious Weeds were prioritized for WRA. PERAL evaluated 31 of these species with its weed risk assessment process, including four that were part of Q-37 market access requests. Of those 31 species, we rated 23 species as High Risk. PPQ risk managers will be proposing some of those species for listing as Federal Noxious Weeds.

- PERAL completed 35 analyses to support access to new overseas markets for U.S. agricultural products, or to expand or retain existing overseas markets. Analyses covered a broad array of commodities, including blueberries, citrus, pome and stone fruit, nuts, ornamental and vegetable seeds, propagative plant material, and potatoes. Target markets included Mexico, Korea, China, Vietnam, and Ecuador, among others.

- DEEP recommended 30 pests for deregulation, and 23 of those were deregulated.

- NPAG completed 33 pre-assessments and 34 reports, which led to 19 pests being regulated and 11 pests being deregulated.

- PERAL completed nine New Pest Response Guidelines.

- PERAL completed 101 pest categorization reports for National Identification Services.

- PestLens analysts produced and distributed 166 articles. In addition, the PestLens web-system was conceptualized, built, tested, and prepared for roll-out.

- The GPDD team added 863 new pests to the database and updated content for 22 high-priority pests, including the addition of 38,800 new distribution records; 32,700 new host records; 2,039 new APHIS documents; and 9,900 new primary sources.

- PERAL provided reviews of 17 draft international and national standards and helped develop standards on diversion from intended use, criteria for determining host status based on available information, and pest risk management.

- Through its Risk Analysis Mentoring Program, PERAL mentored 17 participants from Brazil, Peru, Ecuador, Colombia, Bolivia, Paraguay, Bulgaria, and China.

- PERAL staff delivered a four-day weed risk analysis workshop to 25 participants in Riverdale, MD. PERAL staff also contributed to the Raleigh-based portion of the 2013 Plant Health Systems Analysis course, which was delivered to 20 participants from 14 countries.

**Recent Accomplishments**

**Canada Thistle**

CPHST Ft. Collins continued to support CABI efforts to assess the white rust pathogen *Pustula spinulosa* (formerly *Albugo tragopogoni*) from China as a potential biological control agent for Canada thistle (*Cirsium arvense*). This pathogen has been reported as occurring in the USA on sunflower ‘varieties,’ and physiological ‘races’ of this fungus have been reported in the scientific literature. Further studies are needed to ascertain if the Chinese *Pustula spinulosa* is a different variety or race than the one known to occur in the USA. Field observations suggest that the Chinese *Pustula spinulosa* could be highly specific and damaging to *C. arvense*. Ft. Collins is providing seeds of native U.S. *Cirsium* thistles for use in host specificity testing.

**Diffuse and Spotted Knapweed**

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

**Mile-a-Minute Vine**

*Mikania micrantha* is native to Central and South America, and is considered to be one of the most serious invasive plants in agro-ecosystems in Asia. In 2009, the weed was found in Homestead, FL. CPHST Miami is working with University of Florida and ARS, Ft. Pierce determine the potential for northward spread in the USA and the level of suppression
that extant natural enemies can be expected to exert. The group found multiple arthropod species feeding on the noxious weed that may provide some level of control. Several pathogens collected in the field are currently under laboratory evaluation.

**Rush Skeleton Weed**
In 2012, CPHST Albany initiated a project at the request of the Nez Perce Bio-Control Center in Idaho to develop an artificial diet for rearing *Bradyrhna gilveola* (pyralid moth), which is an agent for biological control of rush skeleton weed, *Chondrilla juncea* (Asteraceae). Four different diets were tested: rice stem borer diet, pink bollworm diet, cactus moth diet and the Hylobius diet developed at CPHST Albany. Our 2013 experiments showed that diet presentation plays a crucial role in feeding induction, with the highest establishment on diets dispensed with ridges. The highest and longest survival rates of *B. gilveola* were obtained on the Hylobius diet; larvae established, molted and fed for 2 months.

**Russian Knapweed**
The Russian knapweed gall midge, *Jaapiella ivannikovi*, was permitted for U.S. release in 2009. A greenhouse-based rearing program for *J. ivannikovi* was initiated at CPHST Ft. Collins in 2011 and continued in 2012 and 2013. Rearing data were collected to document the productivity of the program and to suggest ways to improve its efficacy. In 2011, 2012, and 2013, nearly 3100 *J. ivannikovi* galls were provided for field release at 72 sites in California, Colorado, Idaho, New Mexico, Oregon, Utah, Washington, and Wyoming. We believe that gall midge populations from at least 25% of these sites have successfully overwintered and are established. We will continue rearing and distributions in 2014, likely the final year for this project.

In 2013, FCL initiated a greenhouse rearing program for a second Russian knapweed biocontrol agent, the gall wasp *Aulacidea acroptilonica*, which was permitted for U.S. release in 2008. Our initial field release occurred at a western Colorado site in 2013; we hope to provide adult wasps for field releases in several western states in 2014.

**Yellow Toadflax**
In 2011, CPHST Fort Collins initiated a greenhouse-based rearing program for the yellow toadflax stem-mining weevil, *Melittara janthinus*. In 2011 and 2013, adult weevils from our colony were released at field sites in Colorado and West Virginia. In 2012, we did not provide *M. janthinus* for field release, but instead concentrated on developing a better understanding of adult emergence biology, which was used to optimize rearing efforts. We have discontinued this rearing effort after the 2013 field season. However, CPHST will work with its PPQ partners in providing field-collected *M. janthinus*, as requested, for release across the northern U.S. in 2014.

**Asian Citrus Psyllid**
Since 2009, PPQ has been supporting the development and implementation of mass-rearing and release technologies for the parasitoid *Tamarixia radiata* to help reduce the buildup and spread of ACP populations, particularly in urban areas, and thus minimize the impacts of the ACP-vectored bacterial disease, citrus greening (also called Huanglongbing or HLB) to commercial citrus growers. Rearing (using both greenhouse and field cage systems), release and evaluation efforts, coordinated and funded by PPQ, are now on-going in Florida, Texas, Puerto Rico and California and include cooperators at the Florida Department of Agriculture and Consumer Services, Puerto Rico Department of Agriculture, Texas Citrus Pest and Disease Management Corporation, Texas A&M AgriLife Extension, University of California, and California Department of Food and Agriculture. In FY 2013, the CPHST Mission Laboratory provided approximately 250,000 *Tamarixia* for release in the Rio Grande Valley of Texas, with 147,000 of those being released within 5 miles of the site where HLB was detected in January of 2012. In FY 2013 the CPHST California station provided approximately 150,000 *Tamarixia* in its initial year of field cage rearing for release in the Los Angeles Basin and 11,250 for release in Arizona along its border with California.

**Brown Marmorated Stink Bug**
Since its first detection in Pennsylvania in 2001, the BMSB has now been reported from 40 states and the District of Columbia and has developed into a significant pest of many fruit and vegetable crops, including stone and pome fruits, sweet corn, tomatoes and peppers. ARS has identified several related species of parasitoids in the genus *Trissolcus*, which are generally host specific to stink bugs, as important mortality factors on BMSB in Asia. To expedite host range testing of the natural enemies, CPHST is coordinating a regional approach to collecting and testing native stink bugs, including USDA, and state and university cooperators from DE, MI, OR, MS, FL and CA. Over 20 native stink bug species have been tested thus far against the initial egg parasitoid under consideration *Trissolcus balyomorphae*. Initial results indicate that it has a high degree of preference for BMSB eggs, but it does attack the eggs of some native stink bugs, including those of some predatory species, in no choice experiments.

**Cactus Moth**
CPHST continues to coordinate testing of biological control agents for the invasive Argentine cactus moth *Cactoblastis cactorum*, which poses a serious threat to *Opuntia*-rich areas in the southwestern USA and Mexico. The research team includes scientists from ARS, Florida Department of Agriculture and Consumer Services (FL-DACS), and Mississippi State University. In 2012, permits from PPQ were obtained for shipping the parasitoid *Apanteles opuntiarum* Martinez & Berta (Hymenoptera: Braconidae) from Argentina into quarantine in the US. In March, 2013, approximately 200 *Cactoblastis* larvae infested with *A. opuntiarum* were hand carried from Argentina to the FL-DACS quarantine laboratory in Gainesville, Florida. In June 2013, field explorations were conducted at a number of locations by Mississippi State cooperators in Texas and New Mexico. Colonies of *Melitara prodenialis* and *M. dentata* are currently being reared at FL-DACS and host range testing of the parasitoid is underway. The parasitoid is proving to be very...
difficult to rear as it was discovered that females are heterozygous diploids, while males can be either haploid or homozygous diploids with diploid males typically being sterile. However, initial results continue to indicate the parasitoid is very host specific to *C. cactorum*.

**Grasshoppers**

CPHST Phoenix has been working with ARS in Sidney, MT to assess the efficacy of the entomopathogenic fungus *Metarhizium acridum* (Ma) for grasshopper control. In 2013, was grass treated with Ma and exposed to the spring and fall conditions. Samples were then periodically collected and spore forming colonies were measured to determine survival and infectivity. Research results indicated that the Ma was degraded rapidly by ultraviolet light. It appears that it can survive in the soil / duff but is likely below a threshold that can cause an infection. At the same time an effort to find a domestic source of Ma has been unsuccessful and a similar search in Canada has found no “domestic” Ma. ARS has made contact with a group in New Mexico that has supposedly developed a UV screen for Ma and are testing it in Africa on Green Guard and CPHST Phoenix has been working with strains from Dr. Roberts Lab in Logan UT that are UV tolerant. Methods that may allow field testing in 2014 have been worked out and are awaiting approval by ePermits office.

**Harrisia Cactus Mealybug**

HCM (*Hypogeococcus pungens*) is a severe pest of columnar cacti worldwide and is a major threat to endangered endemic cacti in Puerto Rico and the ornamental industry on the U.S. mainland. Working with cooperators in Puerto Rico, APHIS has identified one parasitoid species (*Leptomastidea sp. nov.*) and one predatory coccinellid beetle (*Decadiomus hughesi*) as candidate natural enemies. Both the *Leptomastidea* and *Decadiomus* are native to the Caribbean basin, and are currently being reared by PRDA for testing and field release in PR. Genetic comparison of the most prevalent parasitoid species, *Leptomastidea sp.*, indicates that it is identical to populations attacking HCM in Barbados and Florida; however a species level identification is pending taxonomic review.

**Imported Fire Ant**

PPQ continues to support and coordinate the IFA Phorid Fly (*Pseudacteon* spp.) rearing and release program. Production of *P. tricuspis* has ceased and that of *P. curvatus* is limited because of their widespread release and establishment. In FY13, multiple releases of *P. obtusus* and *P. cultellatus* were made, with many sites receiving both species. Since 2002, 155 releases have been made.

**Tomato Leafminer**

*Tuta absoluta* is a devastating pest of tomato. In preparation for the pest’s potential spread, CPHST Miami determined that local non-target gelechiid moths in Miami-Dade Co. are attracted to the 2-component lure used by CAPS and the 1-component lure used in Central America and Europe. In addition, experiments conducted in Panama and Miami showed that new adhesives used on trap inserts were as effective in trapping *T. absoluta* while allowing for better visual processing. CAPS modified the U.S. trapping protocols based on these findings. Efforts are currently underway, in cooperation with researchers from Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain, and the University of Panamá to assess the local native enemy community in Florida and Panamá for their potential to control *T. absoluta*. The group discovered the same biological control agent used in Europe’s integrated pest management practices to control the pest was present in Panama.

**Fruit Fly Program**

**Coordinator: Patrick Gomes**

The CPHST Fruit Fly Program supports the APHIS Fruit Fly Exclusion and Detection (FFED) program in meeting its goals to: strengthen detection and response capabilities, conduct preventative sterile fly releases over areas susceptible to fruit fly outbreaks, and ongoing control programs in order to prevent exotic fruit flies from becoming established or spreading within the United States.

In 2013, a Fruit Fly Cross Functional Working Group (CFWG) was created consisting of Policy Management (John Stewart), Field Operations (Shaharra Usnick) and Science and Technology (Pat Gomes and Sarah Marnell). Abbie Fox became the new PPQ Fruit Fly Program Director in Florida.

Efforts centered on providing continued technical and methods support to the field during emergency response quarantine and eradication activities. These include: support to improve fruit fly rearing and emergence techniques; fruit fly aerial sterile releases; release equipment calibration; ground pesticide treatments and operational techniques; enhanced detection; modeling of pest colonization and dispersal; and quality assurance of program resources such as lures, diets, and control pesticides.

CPHST provided important technical and scientific backstopping to the control and eradication efforts that took place during 2013. In California, one quarantine was imposed for oriental fruit fly in Anaheim, Artesia and Cerritos. In Texas, a Mexican fruit fly quarantine covered San Perlita, Mercedes and Harlingen in the Lower Rio Grande Valley. In Florida there was one detection of guava fruit fly but no quarantines.

**Recent Accomplishments**

- Administered fruit fly identification certification test.
- Completed testing on Cereatrap Lure in Florida.
- Completed diet tests to reduce the amount of Torula yeast used in sterile rearing diets to achieve a very significant cost savings to the program.
- Finished mitochondrial work to determine source of *Bactroera* species in California (half of detections are from Hawaii).
• Conducted an analysis of microsatellite markers with University of Hawaii Manoa to determine that B. dorsalis, B. carambolae, B. papayae, B. philippinensis and B. invadens may all be the same species.
• Completed Static ME Spinosad weathering testing.
• Compared quality parameters for sterile flies produced in Hawaii and Guatemala to ensure that both facilities produce quality flies.
• Cooperated on the Compendium of Host Fruit Fly Information. Released MedHOST Version 1.1 – a list of Medfly hosts and references.
• Collaborators completed confirmation work on cherry fumigation in Hawaii.
• Developed a DNA library of Hawaiian populations of Oriental fruit fly, including over 550 DNA samples from flies collected from 2008 to 2012.
• Evaluated dyes used in the Mexican fruit fly program and ranked dyes according to those most easily visible to the identifiers.
• Evaluated cold treatment to mitigate stored insect pests that damaged fruit fly diet and contaminated trapping supplies in Texas.
• Developed shipping, egg bubbling, and handling procedures for Guatemalan-produced Mexican fruit fly eggs.
• Completed testing of antimicrobial disinfectants for control of Mexican fruit fly pathogens (3 Morganella species) that have a detrimental effect on sterile insect mass rearing.
• Conducted calibration and quality control testing on truck-mounted ground release machines for Mexican fruit flies, used when aerial release is not appropriate due to weather conditions, sensitive local areas, etc.
• Evaluated single swath aerial releases for sterile Mediterranean fruit fly in California and Florida and determined that the necessary distribution of flies could be obtained with a reduced number of swaths, leading to a cost savings for the program.
• Completed bio-assay tests which proved that two bacterial species (Providencia rettgerii and P. alcalifaciens) are pathogens of Mexican fruit fly, and can reduce the yield of mass sterile insect rearing.
• Developed a fruit fly rearing monitoring tool (“Dashboard”) to track production data on a daily basis, track the current production level and estimate production levels for the following weeks.
• Used DNA barcode analysis to identify a Mexican fruit fly with aberrant morphological features.
• Implemented an improved adult Mexican fruit fly diet that increases egg production and reduces labor by reducing the number of adult rearing cages needed.
• Confirmed that the current Mexican fruit fly egg infest rate of 60,000 eggs is the optimum infest rate for 6.5-7.0 kg of diet.
• Improved molecular diagnostic methods for pathway analysis for Mediterranean fruit fly and analyzed 316 historical captures in California and Florida.
• Validated and completed a technology transfer of a Kellogg bag covering to replace the current plastic wrap for Mexican fruit fly larvae during rearing – Kellogg bags can be reused up to twenty times, making them more economically and environmentally friendly.
• Used DNA barcoding to identify dead larvae intercepted on clementine shipments from Morocco.
• Completed bioassay testing on methyl eugenol and trimedlure lures in Hawaii and Guatemala.
• Completed trials to evaluate the use of Static Spinosad methyl eugenol (ME) as a possible replacement for Naled Min-U-Gel mixed with ME.

National Plant Protection Laboratory Accreditation Program, Raleigh, NC

Coordinator: Patrick Shiel

The National Plant Protection Laboratory Accreditation Program (NPPLAP) evaluates laboratories to ensure their capability in making accurate molecular diagnostic determinations for regulatory purposes. In addition to ensuring lab capability within PPQ and in other USDA agencies, NPPLAP engages the National Plant Diagnostic Network (NPDN) and State laboratories to increase capacity and proficiency in diagnostic testing among a dispersed laboratory network. NPPLAP fosters the adoption of practices that promote continuous improvement and accreditation standards. NPPLAP works extensively with the NPDN to develop and deploy a functional Quality Management system through partnerships with established accreditation bodies.

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

Recent Accomplishments
• Incorporated quality management principles for planning, production, deployment, assessment and reporting of proficiency tests (PT).
  - Management reviews, Corrective Actions, Customer Feedback.
  - PT report that contains an analysis of testing data to track trends and inform proficiency test group decisions.
• Reformulated the PT program to streamline review, approvals, and timely resolution of testing issues.
- Development of a “Rapid analysis sheet” for automated sample submission and preliminary analysis is in use by the PT program for preparation of reports and issuance of certifications.
- Organized training and provided technical support to promote the NPDPN STAR-D quality management initiatives in plant diagnostics using expertise from CPHST and internationally recognized diagnostic lab testing accreditation organizations.
- Several audit exercises and NPDPN lab reviews under the STAR-D accreditation system were accomplished.
- Developed a process to evaluate and incorporate changes to the USDA work instructions for participant labs. The process:
  - provides a scientifically defensible basis of comparability;
  - saves national reference lab resources that would be needed for constant re-validation of procedures; and
  - changes often incorporate improvements that can be adopted system-wide for increased efficiency and throughput of USDA regulatory samples without sacrificing required assay performance.
- Approved 16 labs and 29 analysts for *P. ramorum* testing.
- Approved 12 labs and 35 analysts for HLB testing.
- Approved 13 analysts for ELISA screening of *Plum pox virus*. The analysts were from 6 separate labs nationwide.
- Participated in the DHS Integrated Consortium of Laboratory Networks.
- Developed a Web portal for administering NPPLAP proficiency tests. This updated approach has helped to streamline deployment, review, and notification of proficiency test programs for diagnosticians and further ensures confidentiality and record integrity.

**National Scientific Technologies Program, Riverdale, MD**

**Coordinator: Laurene Levy**

CPHST's National Scientific Technologies Program (NSTP) assesses and transfers detection, identification, diagnostic, and exclusion tools and technologies to support PPQ programs. The NSTP will ensure that PPQ uses scientifically sound tools and technologies with performance characteristics that enhance PPQ responses to emergency and domestic pests, and pest exclusion and prevention activities at plant inspection stations and ports of entry. The NSTP scans scientific resources for existing and new, innovative tools and technologies for potential adaptation for PPQ programs. Further evaluation and validation is coordinated by the NSTP within PPQ or through cooperation with scientists at government, academic, and commercial industry labs. Selected tools and technologies are then implemented through training and pilot studies coordinated with PPQ leadership and programs.

The NSTP’s current initiative is to strengthen strategic partnerships and facilitate dialogue among the PPQ core functional areas to determine needs and logistics. NSTP also develops strategic partnerships with researchers and operational personnel throughout USDA and other government agencies, especially DHS’ Science and Technology Directorate (DHS S&T) and Customs and Border Protection (CBP); national plant protection organizations; domestic and international academic institutions; and private industry.

**Recent Accomplishments**

- Continued participation on the PPQ Molecular Diagnostic Task Force and on the Methods Sub-group. Distributed a “Survey of international regulatory scientists and officials on the use of molecular diagnostics currently and into the future” to 111 international scientists and officials to understand how other NPPOs are using molecular diagnostics.
- The Molecular Diagnostics Task Force Methods Sub-group developed a matrix of diagnostic methods available and critical method information for the Offshore Pest Information System.
- Participated in a DHS S&T workshop to evaluate new technology that has potential to address requirements in the agricultural screening tools area. Specifically, near term solutions (12-18 months) for field deployable detection systems that can quickly detect and identify agricultural pests of concern (detection of zoonotic, foreign animal diseases, and plant pests). Government subject matter experts evaluated presentations by 9 vendors selected by DHS S&T from over 100 vendors. Three vendors ranked highest and will be further evaluated by DHS.
- Coordinated DHS/PPQ Plant Pest Detection System Project (Gryphon Project) to help guide investments in the research and development of novel plant pest detection systems that will address the needs and requirements of stakeholders in the inspection community. This involved conducting 4 needs interview sessions with 22 participants from PPQ and DHS CBP stakeholders with the DHS Contractor Gryphon Scientific to develop requirements for a plant pest detection system that can assist in the inspection process. The final report findings will be used to develop a proposal for a follow-on project with DHS to develop the inspection system(s) and tools.
- Coordinated, with a DHS S&T Project Manager, the DHS/PPQ project to evaluate volatile organic compound detection systems using surface sampling for methyl bromide from cargo container wood pallets. Using methyl bromide treated wood as a model system, two volatile compound detectors were evaluated in a field experiment at the CPHST Otis Lab. Two systems, the Bruker uRAID and the Oak Ridge National Lab Labrador system, were compared in a head-to-head field trial. The draft report results indicate that the Bruker uRAID device was superior. The final report findings will be used to develop a proposal for a follow-on project with DHS.


Streamlining Technical Procedures for the PPQ Phytosanitary Irradiation Program

Location: CPHST AQI Laboratory, Raleigh NC
Lead Scientists: Laura Jeffers and Woody Bailey
Team Members: Marco Bautista (PPQ Preclearance), Frank Benso (Gateway America; Gulfport, MS), Arved Deecke (Benebión; Matehuala, Mexico)

In the past 5 years, the use of phytosanitary irradiation (PI) on fruit and vegetable U.S. imports has quadrupled (as measured by commodity weight). Despite this growth, PI continues to lag behind the use of fumigation, cold, and hot water as a postharvest quarantine treatment. Obstacles to wider adoption of PI include outdated and overly-conservative requirements for the program.

The historic target endpoint for post-harvest quarantine treatments is mortality. With PI, the treatment endpoint became neutralization (mortality, sterility, prevention of emergence, etc.). The possibility of live quarantine pests on an imported commodity was a paradigm shift for plant health regulators, and the new policies that were established were strict. Over time, the development of adequate safeguards, better data tracking, and a track record of program success warranted a reassessment of PPQ PI program policies from a technical perspective.

At the irradiation industry’s request, CPHST AQI Raleigh started with the examination of process configuration approvals for gamma irradiation facilities (facilities using Co60 as the radioactive source) using batch (only one position in front of the source) or multi-pass (multiple positions in front of the source) systems.

Process configurations are defined by a combination of factors such as article type and size, packaging materials, stacking pattern, bulk density, and density distribution that affect the absorption of radiation and measurement of absorbed dose during treatment. An irradiation treatment facility can have many configurations, each having a different set of treatment criteria. These criteria are determined by their customers, regulatory authorities, and the facility management.

The main concerns of APHIS in the design of process configurations are the assurance of adequate safeguarding and the delivery of the minimum dose ($D_{\text{min}}$). Safeguarding is ensured by the use of approved pest-proof packages. The second major APHIS concern is assurance that $D_{\text{min}}$ will be delivered. This goal is achieved through a two-step process: dose mapping the configuration and developing a simple mathematical model for estimating doses during routine treatments (this model is based on the information obtained in the previous step).

The current APHIS requirement for process configuration approval requires 2 phases of testing, preliminary facility testing and APHIS-monitored testing. Irradiated commodity from these tests cannot be released for distribution in the US and is often destroyed. These additional costs for the exporter/facility make it difficult for irradiation to compete with less expensive quarantine methods (i.e. methyl bromide fumigations). Additionally, if the commodity is to be treated in the United States (Port of Entry Program), then it can be difficult to obtain commodity and packaging materials for the new configuration testing. Again, this leads to additional costs for the exporter/facility and makes irradiation less cost effective than other quarantine methods.

Irradiation facilities submitted several proposals to replace process configuration requirements. Due to the differences in irradiator design, each facility was required to submit a proposal for validation. CPHST AQI Raleigh has validated alternative procedures for three facilities. As an example, the details of one submission for a batch irradiator are offered.

In a batch irradiator, the product only has one position in front of the source during the irradiation process. Because of this, the flux pattern does not change throughout the product as it is irradiated as it does with non-batch/continuous irradiators. Additionally, each product stack is independent from another, and there are no leading or following effects that may affect the absorbed dose of the product, resulting in minimum and maximum absorbed dose locations that are easy to predict.

The facility developed a procedure used to characterize the radioactive source in the irradiator after sourcing and resourcing (i.e. adding or supplementing a radioactive source) and to identify the possible areas where minimum and maximum absorbed dose locations could occur in a new process configuration. The facility provided evidence that the height of the commodity ($y$-axis) does not affect the position of these locations. However, the commodity footprint ($x$ & $z$ axes) can affect these locations. The proposed procedure determines the actual minimum and maximum absorbed dose location as well as the dwell time to achieve the required dose. All new process configurations are tested using this procedure.

To reduce costs even further, validation of alternate process configuration procedures will occur during the initial approval and certification for any new facilities. In the meantime, CPHST AQI has extended this offer to all previously-certified irradiation facilities, and several other proposals are currently under review. CPHST AQI is also assessing policies involving packaging approval, incremental dosing, and re-sourcing validation.
Advanced Molecular Methods for Screening and Confirmatory tests of Citrus Huanglongbing

Location: CPHST Beltsville Laboratory
Project Lead: Wenbin Li
Team Members: John Rascoe and Zonghe Yan

Citrus huanglongbing (HLB), or citrus greening, is a serious citrus disease associated with Candidatus Liberibacter asiaticus (Las), Ca. L. africanus (Laf) and Ca. L. americanus (Lam) and vectored by psyllids Diaphorina citri and/or Trioza erytreae. The bacteria resides in the sieve tube elements of infected plants and also in the vector. Diaphorina vectored HLB-Lam is a heat-sensitive form first reported in Brazil in 2004, and had virtually disappeared five years later. Trioza vectored HLB-Laf is also heat-sensitive and present on the African continent. HLB-Las is the dominant form of the disease and has spread from Asia to the Americas and Africa. HLB-Las was first reported in Florida in 2005, and later in other citrus producing states including Louisiana, Texas, and California.

Before the first report of HLB-Las in Florida, we had developed and validated three sets of 16S rDNA based TaqMan real-time probes and primers, HLBaspr, HLBafpr, and HLBasmpr specific to HLB-Las, Laf and Lam respectively. At the same time we developed plant host (COXfpr) and vector psyllid (WGfpr)-based internal control primer/probe sets for multiplex real-time PCR assays. Since HLBaspr could also react with HLB-Laf, two individual multiplex real-time PCR assays, HLBaspr/COXfpr and HLBasmpr/COXfpr, have been used to cover the three species of HLB bacteria in HLB screening tests since 2005. In FY13, based on validation data, we successfully combined the two assays into one assay HLBasmpr/COXfpr without effecting assay sensitivity or specificity. The combo assay can reduce the cost of the screening test by 50%.

The Asian citrus psyllid (ACP) (D. citri) survey has been used for HLB early detection and management in the United States since 2005 when we developed a method for total DNA extraction from suspect ACP samples. Using this method, DNA extracts could be obtained from 1 to 5 adult ACPs using the Qiagen DNeasy Blood & Tissue kit. Hundreds to thousands of DNA extracts were obtained annually from ACP samples collected by the HLB field surveys in Florida, Texas, and California. Based on our ACP pooling experiments, the ACP sample size per DNA extraction could be increased from 1-5 adults to 1-24 adults without any influence on the DNA extraction efficiency, PCR detection sensitivity, or specificity. This modification in the work instruction can reduce the cost of ACP sample extraction by up to 80% and then could decrease the PCR assay costs for ACP samples by up to 80%.

Conventional PCR assays using the 16S rDNA-based O1/O12c and GB1/GB3, and β operon-based Δ2/J5 primers are being used to create amplicons for sequencing for the federal HLB confirmatory testing program. The real-time PCR assays are more sensitive than the conventional ones. This discrepancy creates inconclusive test results when real-time PCR tests are positive but conventional PCR tests are negative for certain samples collected in HLB surveys. More than 100 additional Las genes were tested for further analysis of the inconclusive results. Five genes were selected as candidate genes for advanced diagnosis. Two of the five candidate genes, chaperonin and heat shock protein (HSP), were further validated. The TaqMan real-time PCR assay based on these two genes can be multiplexed with the COX internal control TaqMan probe/primer set COXfpr for suspect plant samples or with the WG internal control WGfpr for suspect ACP samples. These two additional assays have been added to the U.S. federal confirmatory method for HLB diagnosis to further analyze samples which are positive by 16S rDNA real-time PCR but negative by conventional PCR.

In 2012, PPQ gypsy moth program management requested a spatial risk model for European gypsy moth (EGM) that could be used to prioritize funding allocation based on risk. The first iteration of that model used a GIS weighted overlay approach. It validated very well in the eastern United States and parts of the west against defoliation and treatment area data, but there were some questionable high risk areas. The review of those products prompted a more rigorous modeling approach in 2013 incorporating the use of species distribution models. Species distribution models statistically correlate pest occurrence data against a suite of environmental GIS data layers to produce a prediction surface. There is an abundance of output that can be used from these models to evaluate model fitness, bias, uncertainty, and response to prediction variables. The work includes partnership with the U.S. Forest Service, who had demonstrated prior experience in creating a risk model for emerald ash borer using these methods, and the U.S. Geological Survey, to utilize their modeling software: Software for Assisted Habitat Modeling.

To inform the model, historic gypsy moth detection data was collected from several sources, including APHIS pest survey databases and directly from the states. There was concern of the Slow the Spread data biasing the model fitting process due to its higher contribution in quantity of data. Therefore, the training data was split regionally to parameterize different model components that would be combined at the end. GIS data layers representing different arrival or establishment potentials were created.
Initially, the model was going to create two risk representations, arrival and establishment. However, it was soon recognized that there were different mechanisms of spread occurring in the landscape (natural dispersal and human-assisted movement). Therefore, the arrival model was split into two sub-models of short-range and long-range spread. The same input GIS layers were used for both models, but used regional splits in the detection data for model training. The short range model was explained by distance from previous year detection, whereas long range arrival was explained by a suite of anthropogenic variables such as median household income, traffic volume, road density, address forwarding data, etc. The arrival submodels were combined using maximum value and then inputted as another predictor into the establishment model (i.e. must have an arrival before an establishment can occur). There were challenges with data representativeness in the western U.S., as host species failed to appear as important in the models.

During the model building process, a 10-fold cross validation technique was used to withhold data for validation. Sensitivity analyses were run on the model factors to select the correct level of complexity for best model performance. Finally, all outputs were inspected to evaluate which model performed the best at predicting gypsy moth occurrence data including: response curves; model areas under the curves; confusion matrices; variable importance plots; calibration curves for model bias; and spatial output maps.

Using statistical models over GIS overlay maps grants additional analytical value to pest risk assessments. These methods allow one to directly evaluate model fitness and performance using a suite of standardized outputs. The final prediction maps can inform survey sample design in the next field season by targeting highest risk areas. Additional outputs can be used to evaluate model uncertainty, and to target sampling in areas where less is known about gypsy moth response to those conditions. And finally, one can induce a machine learning element by utilizing information learned from the previous field season into the next model, making the model “smarter” and reducing uncertainty over time.

**Mass Production and Releases of Tamarixia radiata**

*Tamarixia radiata*, a species-specific ectoparasitoid of the Asian citrus psyllid (ACP), *Diaphorina citri*, was imported from Pakistan and permitted by the PPQ Permitting Unit for field release in Texas. Methods to produce large numbers of *T. radiata* were tested and developed for mass-production of the beneficial insects. Releasing large numbers of *T. radiata* to establish resident populations can help suppress populations of the ACP in order to slow the spread of *huanglongbing* (HLB), or citrus greening disease. Protocols developed for mass production using greenhouses, yielded 869,618 parasitoids which were released at 397 sites throughout the Rio Grande Valley. The highest concentration, 333,606 parasitoids, was released within a 5-mile radius of the HLB find in San Juan, Texas. Current efforts are being focused on a new site of infection in Mission, Texas.

**Targeted Areas in the Lower Rio Grande Valley**

Our target areas in the urban environment have focused on former citrus groves that have been converted into recreational vehicle parks (RV parks) for winter residents. Some properties measure as much as 30 to 40 acres in size and may contain hundreds of citrus trees which typically go untreated. At the RV parks, we receive much support and enthusiasm from residents. They are very curious about what we do and often like to watch as we gather data on various parameters and conduct releases of the beneficial insects from vials.

**Open vs Closed Releases**

Both open and closed (or caged) releases were conducted and used to assess establishment and efficacy. Most of our beneficial insects were released openly on trees harboring Asian citrus psyllid nymphs. We conducted closed releases as part of our assessments to document the establishment of the beneficial insects. Mortality of psyllids in closed experiments (in fine-mesh cages) was 4.4% without parasitoids and 75.3% when parasitoids were introduced. Parasitism per se explained 13.8% of this mortality. Further investigations into the unexplained mortality of ACP nymphs were explored.

**Host-Feeding / Host-Mortality Studies**

We conducted visual observations examining the behavior of female parasitoids (n = 30) for one-hour periods in arenas with suitable hosts. Data indicates that females will mount 3.1 ± 0.5 nymphs per hour. The parasitoid will either oviposit on the ventral side of the nymph (36.5% of the time) or probe the nymph on the dorsal side (63.5% of the time). After probing, the parasitoid will either walk away (87.9% of the time) or host feed (12.1% of the time). Host feeding was documented at 0.43 ± 0.1 nymphs per hour. All nymphs that were fed on eventually died. Further methods are still being developed and explored to help reduce both ACP populations and the incidence of citrus greening disease.

**Field Insectary Cages**

In collaboration with Texas A&M Agrilife Extension Service and the local Master Gardeners of Texas, we began work on our field insectary cage study across the Lower Rio Grande Valley of Texas.

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**Evaluating the Biological Control of Asian Citrus Psyllid in the Lower Rio Grande Valley of Texas**

**Location:** CPHST Mission Laboratory  
**Lead Scientist:** Dan Flores  
**Team Members:** Matt Ciomperlik, Andrew Parker, Rupert Santos, Fidel Mendoza, Mayra Rangel, Gilbert Salazar, and Eustorjio Rivas
Valley. Master Gardeners is a volunteer program that advises and educates the public by working through extension offices. Partnership with the program and a high level of homeowner support has allowed us to foster the propagation and mass release of *T. radiata*. Figure 1 shows the installation of a frame used to cage psyllids and *T. radiata* on citrus trees. This lemon tree measured 15 feet in diameter and 13 feet in height before being hedged. Hedging the tree results in the propagation of new growth, also known as “flush”. We allowed the psyllid to lay eggs for two to three weeks after hedging (during the flush cycle) before introducing the beneficial insects. At the time of introduction, the mesh screen was placed over the frame for about 8 weeks to allow the beneficial insects to build up. Once the populations have built up, the mesh screen and cage frame are removed to allow the beneficial insects to freely disperse. Between February and September 2013, twenty six field insectary cages were installed in residences of the south Texas community for production of the beneficial insects. Over 12,000 insects were produced per caged tree. In addition, the large cages allowed for outreach opportunities to be conducted within the community. These efforts will be enhanced in the next fiscal year.

**Mexico Collaboration**

Though a cooperative agreement developed with APHIS International Services and the University of Texas – Pan American, the Mission Lab began supplying parasitoids for release along the Mexico side of the US/Mexico border in order to create a biological barrier. Over 175,195 parasitoids of *T. radiata* were released in the Reynosa - Rio Bravo area in the months between June and September 2013 at over 45 locations. Seventeen of these sites had the local *T. radiata* present at levels of 10.78% parasitism. Two weeks after each release, data was collected at the release sites for assessment of establishment and parasitism. Establishment was confirmed at all release locations. Overall percent parasitism increased to an average of 62.39% parasitism at all the release sites. We are continuing to make additional releases.

**Development of Radiofrequency (RF) as Part of the Dielectric Heating Standard for Solid Wood Packing Material (SWPM)**

**Location:** CPHST Otis Laboratory  
**Lead Scientist:** Ron Mack  
**Team Members:** Peggy Elder  
**Cooperators:** John Janowiak, Brad Gething and Kelli Hoover (Penn State University); Adnan Uzunovic (FP Innovations, Forintek, Canada)

The solid wood packing material (SWPM) pathway has introduced a number of devastating pests to forested landscapes around the world. In North America, concerns over pest introductions via SWPM lead to the Solid Wood Packing Material from China Interim Rule in September 1998, an emergency measure issued by the U.S. government that stated “all wooden packing material being shipped from China to the United States must be heat treated or fumigated with methyl bromide prior to departure from China” (USDA 1998c). International concern then lead to ISPM-15, a universal standard that required treatment of SWPM using conventional heat (56°C for 30 minutes throughout wood profile) or methyl bromide fumigation. The standard was adopted by the Commission on Phytosanitary Measures (CPM) in March, 2002, with subsequent modification in 2006 (fumigation schedule) and revision in April, 2009 (addition of debarked wood standard). Reliance on methyl bromide in the Interim Rule and ISPM-15 was problematic due to ozone depletion concerns. For example, the Montreal Protocol, a universal treaty that originally went to signature in 1987, has a stated goal of eliminating the production and use of methyl bromide and other ozone depleting chemicals along a specified timetable.

The phase-out of methyl bromide gave urgency to the need for suitable replacement treatments. The International Plant Protection Convention (IPPC) Secretariat initiates the process of calling for new treatments by tasking IPPC members for priorities regarding phytosanitary treatments for international standards as required. The prioritization criteria are then used by National Plant Protection Organizations and Regional Plant Protection Organizations to submit requests for priority treatments (IPPC 2014). Requests are forwarded to the Technical Panel of Phytosanitary Treatments (TPPT) for review and recommendation on priorities for development of phytosanitary treatments as ISPMs. Once the treatment priorities have been established by the TPPT, the IPPC Secretariat issues a formal call for submissions of supporting data for those treatments. Plant Protection Organizations are responsible for forwarding the data submission packages under guidance from ISPM-28:2007 (Phytosanitary Treatments for Regulated Pests).

The dielectric heat treatment of SWPM was submitted to the IPPC Secretariat in response to the 2006 call for treatments. Dielectric heating technically refers to heating using energies in the microwave and radiofrequency bands on the electromagnetic spectrum. The original submission was entitled “Microwave Irradiation of Wood Packing Material”, given that all supporting data were developed on targeted pests using experimental microwave ovens. The TPPT found that the original dielectric submission had to further demonstrate treatment efficacy at a Probit-9 level for pinewood nematode. These supporting data were provided in 2009, and the TPPT concluded that microwave irradiation treatment of wood (a minimum temperature of 60°C for 1 minute throughout the profile of the wood) has been shown to be an effective treatment against the pests listed in Annex 1 to ISPM 15:2009, and should be approved for wood not thicker than 20 cm (IPPC 2010).

Radiofrequency experiments were initiated in 2009 to combine with microwave and validate the entirety of the dielectric spectrum for treatment efficacy. Under the guidance of several international technical panels, namely the TPPT, Technical Panel of Forest Quarantine, and the International Forest Quarantine Research Group, a testing protocol of
suitable rigor was put in place to evaluate the efficacy of radiofrequency. A broad spectrum of pests and pathogens was identified for testing, and the workload was split among cooperating researchers at the Otis Laboratory, Pennsylvania State University, and FPInnovations, Forentek, Canada. FPInnovations conducted research on lethal temperature for pinewood nematode, *Bursaphelenchus xylophilus*. The Otis Lab and Penn State were jointly responsible for data development on Asian longhorned beetle (ALB) and emerald ash borer (EAB). Tests on target organisms were designed to validate the DH schedule of 60°C for 1 minute hold time. Given the variability and complexity of wood and the short treatment time, particular attention was focused on heating uniformity through thermal mapping.

Otis contracted with PSC laboratories, a subsidiary of C.A. Litzler Co., Inc. to design and construct a portable RF oven to be shared cooperatively among the radiofrequency research teams. The 15 Kw oven operating at 19 MHz was designed for lab scale testing of small wood pieces between an upper and lower electrode that provided a treatment volume of 24” x 24” x 14” in height. For the ALB study, the radiofrequency machine was placed in Worcester, Massachusetts, in an established ALB quarantine zone. Infested material was collected at another ALB infestation in Bethel, OH and shipped to Worcester for testing. The Bethel material was preferred due to the greater larval incidence in the collected wood. A minimum of 60 infested bolts were tested for each of the following target temperatures: 35, 40, 45, 50, and 55°C along with control set. The treatment at 55°C resulted in 100% mortality of ALB larval stages in the wood. Probit modeling predicted a treatment of 59.4°C at .9999 probability and 95% confidence at one minute hold time. The EAB study was designed similarly and was conducted in Brighton, Michigan. A minimum 60 infested bolts were treated at the following target temperatures: 35, 40, 45, 50, and 55°C along with control set. The treatments at 50°C and 55°C resulted in complete mortality of all EAB life stages in the wood. Probit modeling predicted a treatment of 60.6°C C at .9999 probability and 95% confidence at one minute hold time. Probit modeling was chosen for both ALB and EAB due to the relatively low numbers of life stages encountered and analyzed. Results on the study conducted by FPInnovations using the same radiofrequency oven found that all wood samples that met or exceeded 56°C lethal temperature for the required one minute hold time resulted in 100% mortality on pinewood nematode (Uzunovic, 2013).

The results obtained for efficacy testing on pests using radiofrequency are consistent with the result obtained using microwave, thus validating dielectric treatment across the full spectrum. A revision to ISPM-15 Annex 1 in April, 2013 allowed for inclusion of dielectric heating as the first alternative treatment accepted for SWPM by the CPM of the IPPC. Additional work planned in conjunction with Penn State will involve considerations for proper scale-up of dielectric treatment. Large scale experiments are planned for batch treatments that optimize heating uniformity, as well as plans for written guidelines for potential operators of dielectric equipment.

**References**


### Softwood Ammunition Boxes of the United States Department of Defense as a Pathway for the Pinewood Nematode and Other Pests of Quarantine Concern to the European Union

**Location:** Plant Epidemiology and Risk Analysis Laboratory (PERAL)

**Lead:** Heike Meissner

**Members:** Leah Millar¹, Edward Jones¹, Laney Campbell², Dawn Holzer³, Tyrone Jones³, Andrea Lemay⁴, Weimin Ye⁵, Patrick DeWald⁶, John Fitzpatrick⁶, Jared Shuppy⁶, Hugh “Pat” Murray⁶, Eugene “Mike” Ivankoe⁶; numerous PPQ-FO inspectors, DoD depot personnel, and NCDoA lab staff.

¹PERAL, ²PPQ-Field Operations, ³PPQ-Policy Management, ⁴APHIS-Policy and Program Development, ⁵NC Dept. of Agriculture, ⁶Department of Defense

This analysis was requested by the United States Department of Defense (DoD). The DoD has stockpiles of ammunition—packed in wooden boxes—stored in various depots throughout the United States. There is an ongoing need to move this ammunition to the European Union (EU), which requires that the associated wood boxes be treated in accordance with the International Standard for Phytosanitary Measures (ISPM) No. 15 to reduce the possibility of inadvertently spreading wood pests, especially the pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Nematoda: Parasitaphelenchidae). By September 2007, all new DoD WPM was treated according to EU phytosanitary regulations. However, some 1,200 metric tons of wood boxes that have been in storage since before that time are not compliant with ISPM No. 15.

Treatment of ammunition boxes is difficult, expensive, and time-consuming because it requires the unloading of explosive materials from the boxes, which necessitates special safety measures. To avoid the costs and logistical challenges associated with this, the DoD decided to request an exemption from the EU’s treatment requirements, arguing low pest risk of the boxes.
To provide the scientific basis for this exemption request, PERAL led a pathway analysis effort, including a pest survey of DoD ammunition boxes. Specifically, PERAL a) led a meeting with an EU technical advisory panel to scope out the project, b) developed the statistical design for an extensive pest survey of DoD ammunition boxes, c) developed detailed guidelines for the PPQ and DoD staff carrying out the survey work, d) coordinated nematode extraction and identification efforts with the Nematology Laboratory of the North Carolina Department of Agriculture, e) produced a pathway risk analysis, incorporating the results of the pest survey as well as biological and pathway information, and f) led the preparation of a manuscript describing the results of the pest survey for publication in a peer-reviewed journal. The entire undertaking was a two-year collaboration involving all PPQ core functional areas, in addition to APhIS-Policy and Program Development, the North Carolina Department of Agriculture, the USDA Forest Service, and several different units within the DoD. In total, over 50 people participated in the project.

PERAL served as the liaison between the collaborating agencies and played a key role in project management. As a result of competent project planning and execution, all survey, nematode extraction, and pathway analysis work was completed on time, on budget, and without logistical, technical, or inter-personal problems. The PERAL pathway analysis demonstrated satisfactorily that the DoD ammunition boxes in question present a negligible pest risk to the EU. In response, the EU has formed a working group to develop the requested exemption. Once finalized, this exemption is expected to save the DoD millions of dollars in treatment costs, in addition to minimizing delays of critical ammunition shipments and avoiding treatment-related environmental impacts. Several PPQ project members received awards from the DoD in recognition of their outstanding work on this project. The results of the pest survey were published in the EPPO Bulletin. The project also contributed to a second manuscript, published by the North Carolina Department of Agriculture. The many positive working relationship formed across agencies may prove helpful to PERAL in the future. Overall, this project serves as an example of a truly collaborative, cross-functional, and technically successful PPQ effort of potentially high impact.

**Initial Comparison of the Standard Organophosphate Malathion and a New Extended Residual Formulation for Control of Rangeland Grasshoppers**

**Location:** CPHST Phoenix Lab  
**Lead Scientists:** Larry Jech and Nelson Foster  
**Team Members:** Chris Reuter and Lonnie Black

Rangeland pests, when requested, are controlled by PPQ with a limited spectrum of chemistry, including organophosphates, carbamates, and insect growth regulators. Malathion, a short residual organophosphate, has been widely used as an ultralow volume treatment for grasshopper and Mormon cricket to protect rangeland; and was often used to control older, adult and migrating rangeland pests when there was no threat of continued hatch and before oviposition was initiated.

A manufacturer’s reformulation, to extend the residual activity of the organophosphate, was field tested near Bayard, Nebraska. The manufacturer, Cheminova, added 1.47% gamma-cyhalothrin to the formulation to extend the field life and provide a more economical and longer lasting treatment. A reduced agent-area treatments (RAATs) application incorporates untreated areas that are usually equal to the swath of the aircraft. This has the effect of reducing the insecticide impact on non-target arthropods while providing relief from pests to the rangeland resources. Because malathion has a short residual, when used in RAATs application the airplane is calibrated for 75 feet and the malathion is applied at a swath spacing of 100 feet, whereas the RAATs application for carbamates and growth regulators are equal. This has an impact on the cost benefit ratio and timeliness of treatments. If the new formulation has a longer residual, the RAATs treatment and skip could be made equal (swath = skip). The following test evaluated the claim of longer field residual activity.

The malathion with and without the gamma-cyhalothrin was applied in unreplicated plots and sampled for grasshopper population change. At the same time five untreated grasshoppers were caged in ten buckets on treated plots with suitable treated hosts. Mortality was followed daily. Additional grasshoppers were caged on day 2, 4, and 6 post treatment on the plots as the malathion aged under natural field conditions. The results indicated that the addition of the gamma-cyhalothrin to malathion did enhance the field activity against grasshoppers. Tests were terminated after 6 days. Examination of the data indicates that the final test date may need to be extended to capture the extended residual field life. The longer field life would allow testing of the formulation in a normal (treat a swath, skip a swath) RAATs pattern that would enhance the cost benefit ratio and provide a new treatment for use against grasshoppers that are too old for the diflubenzuron treatments.

**Biological Control of Russian Knapweed**

**Location:** Fort Collins Laboratory, Biological Control Program  
**Lead Scientist:** Rich Hansen  
**Team Members:** Lynn Morales, Tara Costanzo

Russian knapweed, *Rhaponticum repens* (Asteraceae), is a perennial plant native to Asia that was introduced into North America in the late nineteenth century, probably as a crop seed contaminant. It has become a widespread weed in the western United States, where it infests rangeland and riparian areas as well as agricultural fields, pastures, and a variety of other disturbed habitats. Russian knapweed forms dense
monocultures that displace native and desirable crop and forage plants. It is distasteful to most grazing mammals and may be toxic to horses.

Cultural and chemical control options can be effective against some Russian knapweed infestations, but many sites are large, remote, and of relatively low economic value. In the late 1990s, PPQ and Federal, state, and Canadian partners initiated an effort to develop classical biological control agents for this weed. The first two insects permitted for U.S. release were the gall wasp *Aulacidea acroptilonica* (Hymenoptera: Cynipidae), in 2008, and the gall midge *Jaapiella ivannikovi* (Diptera: Cecidomyiidae), in 2009. The gall midge induces gall formation on terminal and lateral buds of Russian knapweed shoots, while the gall wasp generally forms stem galls. Both agents are highly host-specific, and present little risk of nontarget native or crop plant attack in North America.

In 2011, the Fort Collins Laboratory (FCL) initiated a greenhouse-based rearing effort to provide *J. ivannikovi* for field releases in western states. The midge has been released at 72 sites in eight states (California, Colorado, Idaho, New Mexico, Oregon, Utah, Washington, and Wyoming) from 2011 through 2013. At least 25% of the 2011 and 2012 sites have established *J. ivannikovi* populations. We will continue monitoring *Jaapiella* release sites in 2014, and initiate releases in at least two new states. In 2013, FCL initiated a greenhouse rearing program with *A. acroptilonica*, using insects collected at Montana field sites by Montana State University collaborators. Galls produced in this first year will provide adult wasps for 2014 field releases in selected western states, and continuation and expansion of the FCL rearing effort. We also provided some wasps for release at two western Colorado sites in 2013. FCL has worked with many PPQ, Federal, state, university, local, and tribal partners in releasing and monitoring Russian knapweed biocontrol agents.

Impact assessments with field populations of *Jaapiella ivannikovi* are presently premature. However, it seems likely that both *J. ivannikovi* and *A. acroptilonica* will reduce Russian knapweed flowering and seed production and may reduce aboveground biomass, but will probably not cause plant mortality. Perhaps these agents will be suited for integration with other management tools, such as planting of competitive grasses.

**Improvements to Process Control, Data Management and Analysis through the Use of the Mass-Rearing Monitoring Tool (aka “The Dashboard”)**

**Location:** Fruit Fly Program, Guatemala  
**Lead Scientist:** Pedro Rendon

CPHST Guatemala cooperators working closely with the rearing staff at San Miguel Petapa developed a mass-rearing monitoring tool (also known as “The Dashboard”) using Excel that provides program managers with a very useful tool for monitoring production processes on a daily basis. The Dashboard establishes key metrics such as the milliliters of eggs produced, number of trays infested, larval recovery (liters of larvae per kilogram) and liters of larvae or pupae produced each day. Rearing staff enter these metrics into the spreadsheet each day. From this, the spreadsheet calculates the millions of pupae that will be produced and when they will be available for irradiation. The Dashboard generates graphs showing daily values compared to mean values and standard deviations. As a result, minor changes or modifications to improve rearing processes can be assessed with precision and accuracy. The Dashboard can be used to track standard as well as genetic sexing strains for fruit flies. The Dashboard underwent one year of beta testing at the Mexican Fruit Fly Facility in Guatemala before it was transferred in April 2013 to the Edinburg Mexican Fruit Fly Rearing Facility in Texas for use and now is being considered for adoption as an international process control standard by the Joint FAO/IAEA Division for Nuclear Techniques in Food & Agriculture. Apart from tracking daily production, the Dashboard also keeps track of actual use of diet materials and on-hand inventories.

**Requirements for a Novel Plant Pest Detection System**

**Location:** National Scientific Technology Program  
**Lead Scientist:** Laurene Levy

**Team Members:** PPQ S&T: Mike Hennessey, Russ Bulluck, Talitha Molet, Philip Berger, Lisa Mosser, and Amy Roda; PPQ Policy Management: Joseph Cavey, Jeffrey Grode, Ron Komsa, Michael Simon, Calvin Shuler, Evelia Sosa, and William Thomas

**PPQ Field Operations:** Marla Cazier-Mosley, David Farmer, Roger Holman, and Katherine Hough

**Department of Homeland Security (DHS), Customs and Border Protection (CBP), Agriculture Programs and Trade**  
**Liaison (APTL):** Kevin Harriger, Mikel Tookes, Donka Weaver, Josue Ledezma, Petrina Evans, Nikki Thomas, Eunett James-Mack and Robin Wall.

**Cooperators:** Angela Ervin, DHS Science and Technology (S&T) Directorate; Rocco Casagrande and Anna Kushnir, Gryphon Scientific, LLC.

The goal of this DHS-funded project is to help guide investments in the research and development of novel plant pest detection systems that will address the needs and requirements of stakeholders that conduct inspections. In order to improve the efficacy and throughput of inspections, PPQ and DHS S&T cooperated to gather the requirements and scan the scientific horizon for technology. DHS S&T contracted with Gryphon Scientific to develop the requirements for a novel plant pest detection system.
The project objectives included:

- determining the stakeholder requirements for detection technology by interviewing stakeholders;
- identifying the gaps between existing detection technologies and stakeholder requirements;
- identifying current and emerging technologies;
- defining parameters for a notional detection system based on stakeholder requirements; and
- providing recommendations on technology and how to address the gaps through further research and development.

PPQ, DHS, and Gryphon compiled a list of 22 subject matter experts (16 from PPQ and 6 from CBP) and developed a series of interview questions. Gryphon staff conducted the interview in 4 sessions with the PPQ and CBP experts that were asked to describe existing inspection workflows/processes, and define requirements for a detection system. The participants were asked their desired concepts of operation; performance characteristics, physical parameters, and cost limitations for the detection system. Following the interviews a site visit to the Newark port of entry and Linden plant inspection station helped the Gryphon team outline the inspection needs and limitations. The PPQ and CBP project team detection system requirements were:

- 95% accuracy (false positive and negative rates at 5% or below),
- if possible, detection sensitivity of one organism,
- find the pest in passenger luggage within seconds and in cargo within 1 hour,
- find plants in non-plant cargo and if possible detect if to a family level,
- be portable weighing 3-5 pounds,
- be powered by a battery that maintains an 8 hour charge and cost between $1,000-20,000 (per inspector) or $15,000-50,000 (per work unit), and
- requires less than one week of training.

Based on the technology requirements determined the Gryphon team reviewed scientific articles, government reports, device brochures (for commercially-available technologies) and searched patents. The technology identified were then assessed for specific parameters that included cost, sensitivity, error rate, pests that could be detected, flexibility, physical parameters (size, weight, portability) and technology readiness level.

Four technology areas were identified that matched the requirements: molecular detection techniques, volatile detection, acoustic detection, and imaging. Molecular detection was determined to be more useful to Identifiers following detection and was shelved as a follow-on project. Volatile detection can detect pests by sampling the headspace in a shipping container or crate and detect volatile organic compounds produced by stressed plants or by the pests and pathogens themselves. Acoustic detection can detect sounds and vibrations produced by wood boring, tunneling or feeding adult insects and larvae. If the insect is active the detection could happen in real time and stand-off probes could “listen” to whole containers/boxes or crates. Acoustic detection measures sound so insects need to be alive and pathogens can’t be detected. The final technology is imaging including thermal imaging, near-infrared, hyperspectral and X-ray. Thermal imaging requires that the pest give off enough heat within the sample/shipment to be detected by the imager. It was determined that insects in shipments may not give off enough heat to be detected. Near-infrared imaging (spectroscopy) is already in use in seed sorters to detect pathogens and invasive weeds in grain and could increase the effectiveness and efficiency of inspection of seed or grain imports. Hyperspectral imaging scans whole plants/fruit; however it can’t penetrate boxes and can only assess the outside of the plant material. This technology may also be able to scan containers. Finally, X-ray imaging is currently in use by both PPQ and CBP.

The final assessment recommended four technology areas: volatile detection, acoustic detection, near-infrared seed sorters, and hyperspectral imaging. The project report provides in-depth evaluation and inspection scenarios that assisted the Gryphon team in evaluating the technologies. DHS has funded a follow-on Phase II project where Gryphon will outline the validation and verification steps that would be required to thoroughly test each potential technology identified in Phase I (described above) before it can be deployed. This can assist in prioritizing technology development for inspections and provide a cost/benefit assessment to guide PPQ investments. The Phase II project will also identify technologies that can assist in plant pest identification which was put aside in the Phase I project.
Publications

The following is a list of scientific articles published by CPHST employees in fiscal and calendar year 2013.


**Publications by Cooperators**

The following is a list of additional publications by CPHST cooperators based on cooperative agreement work.


Scientific Meetings

CPHST scientists participate in national and international scientific meetings, workshops, and conferences in order to promote the exchange of the latest scientific information on plant protection methods. The following is a list of such meetings attended in 2013.

- American Phytopathological Society Annual Meeting
- American Society for Horticultural Science
- American Society for Virology
- FBI Laboratory Chemical, Biological, Radiological, and Nuclear Sciences Unit Partner Lab Symposium
- California Citrus Pest and Disease Prevention Committee Meeting
- California Citrus Research Board – New Technologies Conference
- Entomological Society of America Annual Meeting
- Entomological Society of America Eastern Branch Annual Meeting
- Eco-stats Symposium
- EPA International Decontamination Research and Development Conference
- Imported Fire Ant and Invasive Pest Ant Research Conference
- IPPC Technical Panel for Diagnostic Protocols
- International Citrus Congress
- International Plant Protection Convention Technical Panel on Phytosanitary Treatments
- International Atomic Energy (IAEA) Cooperative Research Program “Resolution of cryptic species complexes of tephritid pests to overcome constraints to sterile insect technique application and international trade”
- IAEA General Conference
- International Conference on Controlled Atmosphere and Fumigation
- International Conference on Globalization, Climate Change, and Technological Convergence
- International Irradiation Association International Meeting for Radiation Processing
- International Research Conference on Huanglongbing
- Methyl Bromide Alternatives and Emission Reductions Annual Conference
- National Grasshopper Management Board Meeting
- National Cotton Council Technical Action Committee Meeting
- National Gypsy Moth Program Review
- National Stakeholders Conference on Honey Bee Health
- North American Plant Protection Organization (NAPPO) Symposium on S&T Tools for Phytosanitary Use
- North American Invasive Plant Short Course
- Northern Rockies Invasive Plant Council
- Opportunities in Phytosanitary Irradiation for Fresh Produce Workshop
- Subtropical Agriculture and Environments Society Meeting
- USDA Interagency Forum on Invasive Species
- USDA Technical Advisory Group for Biological Control Agents of Weeds
- Wyoming Weed and Pest Council
Appendix A. Funded Projects for Fiscal Year 2013

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<tr>
<th>Funding Category</th>
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<td>Imported Fire Ant</td>
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<td>Light Brown Apple Moth</td>
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<td>Cotton Pests</td>
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<td><strong>Total</strong></td>
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Funding lines for CPHST in fiscal year 2013 includes allocated and user fee funds of about $32 million and Farm Bill Section 10201 funds of $11.5 million.

### AQI Laboratory and Biloxi Station
- Evaluating Biomarkers for Verifying Successful Phytosanitary Irradiation Treatments in Multiple Atmospheres (University of Florida)
- Improving Techniques for Detection of Prohibited Plants and Invasive Pests at Ports of Entry (University of Florida)
- Lure Shelf Life and Quality Assurance Determination (USDA-ARS)
- Assessment of Remote Sensor Technology and Portable Gas Chromatography As an Agricultural Screening Tool (DOE-Oak Ridge National Laboratory; University of Florida)
- Outsourcing Pesticide Residue Analysis on APHIS Eradication and Suppression Program Samples (USDA-AMS)
- Development of Grass Sod Treatments for IFA Quarantine (Auburn University; University of Arkansas)
- Development of IFA Quarantine Treatments for In-field and Balled-and-Burlapped Nursery Stock (USDA-ARS; Tennessee State University)
- Biological Control of the Imported Fire Ant using Phorid Flies: Cooperative Rearing and Release Program (Florida Division of Plant Industry; USDA-ARS)
- Development of Rapid IFA Assay Kit (ID) and Species Specific Surveillance Trap (USDA-ARS)

### Beltsville Laboratory

#### Core Projects
- Development of molecular assays for oomycetes and *Phytophthora* spp. of regulatory concern
  - *Phytophthora kernoviae*
- Development and delivery of PPQ proficiency panels for PPV, *P. ramorum* and HLB
- Development of molecular assays for fungal pathogens of regulatory concern
  - Potato wart
  - *Harposphora maydis*
- CANARY development and implementation
  - *Phytophthora*
  - *Ralstonia*
  - *Citrus leprosis virus*
  - *Citrus variegated chlorosis*
  - *Potyviruses*
- Development and adaptation of molecular assay for viruses and viroids on concern in foreign germplasm
- PPQ confirmatory diagnostics
  - *Candidatus Liberibacter* (HLB-Citrus Greening)
  - *Candidatus Liberibacter* in Asian citrus psyllid (HLB-Citrus Greening)
  - *Phytophthora ramorum*
  - Plum pox virus (PPV)
  - *Xanthomonas axonopodis pv. citri* (Citrus Canker)
  - *Ralstonia solanacearum* R3B2
  - Potato cyst nematode (PCN)
  - Phytoplasma
  - *Elsinoe australis* (Sweet Orange Scab)
  - Guignardia citricarpa (Citrus Black Spot)
  - *Capsicum chlorotic virus* (CaCV)
  - *Cucumber green mottle mosaic virus* (CGMMV)
  - *Citrus leprosis virus* (GLV)
  - *Phytophthora hedraiandra*
  - *Phytophthora multivora*
  - *Phytophthora fallax*
  - *Phytophthora tentaculata*
  - *Xanthomonas citri pv. mangiferae*
  - *Neofusicoccum crypto-aurale*
**Farm Bill Funded Projects**

- Molecular Diagnostic Methods for *P. ramorum* and *Sclerotinia* species of Biosecurity Concern
- Priority Research & Development for Citrus Health Response Program Pests
  - HLB, CVC, GiLV, CBS and SOS
- Online Identification Tools to *Phytophthora*: Lucid Key, Tabular Key and Sequencing Analysis
- Diagnostics of *Pseudomonas syringae* pv. *actinidiae*, causal agent of bacterial canker of kiwifruit.

**Fort Collins Laboratory**

**Biological Control**

- Biological control of Russian knapweed
- Biological control of field bindweed
- Biological control of yellow toadflax and garlic mustard
- Biological control of hoary cress
- Biological control of hound’s-tongue
- Biological control of dyer’s woad and perennial pepperweed
- Biological control of hawkweeds
- Risk assessment and monitoring of target and nontarget plant utilization by the hound’s-tongue root weevil *Mogulones crucifer* in northern Washington and Idaho (University of Idaho)
- Development of a database: biocontrol agents of insect pests released in the United States (Colorado State University)

**Decontamination and Waste Disposal**

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

**Digital Identification Tools and Resources**

- Grasshoppers of the Western United States, Edition 4 (Chadron State College, Nebraska)
- Morphological and molecular diagnostic tools for *Lepidoptera* larvae intercepted at US ports of entry (Farm Bill-funded, Colorado State University)
- Identifying and cataloguing globally available resources to support off-shore and pest identification (Colorado State University)
- Developing *Lepidoptera* screening aids for CAPS (Farm Bill-funded, Colorado State University)
- Developing digital pest identification tools for off-shore and port personnel (Colorado State University)
- CapsID: web-based resources for pest screening (Colorado State University)
- Digital imagery for pest screening, detection, and identification (Farm Bill-funded, Colorado State University & University of Georgia)
- Microlep on Solanaceous plants (Farm Bill-funded, Division of Plant Industry, Florida Department of Agriculture and Consumer Services & Arizona State University)
- Hispine beetle multi-media off-shore and port identification support aids (Smithsonian Institution, National Museum of Natural History)
- Safeguarding against scale insects pests: a digital tool for training screening and identification (Farm Bill-funded, Plant Pest Diagnostics Branch, and California Department of Food and Agriculture)
- Mobile apps for citrus pest and disease screening (University of Florida & Texas A&M University & University of California)
- Developing IDpic’s contributor interface (Farm Bill-funded, University of Georgia)
- Resources for off-shore and port invasive ant detection and identification (University of Illinois)
- Identification of flat mites (*Acari: Tenuipalpidae*) of the World: *Brevipalpus* and *Raoiella* (Farm Bill-funded, University of Maryland)
- Online identification tools for Phytophthora: Lucid key, tabular key, and sequencing analysis (Farm Bill-funded, University of Maryland)
- Identification tool to invasive *Diabrotica* species (Farm Bill-funded, University of Maryland & Systematic Entomology Laboratory, Agricultural Research Services)
- Identification tools to longhorn wood boring beetles (Farm Bill-funded, University of New Mexico)
- A resource for wood boring beetles (University of New Mexico)
- Lucid Mobile apps for Android and iOS phones (University of Queensland)

**Spatial Technologies**

- Strategic planning and policy development for APHIS PPQ geospatial resources
- Nationwide European gypsy moth predictive model to assist resource allocation and surveillance
- Nationwide Asian gypsy moth predictive model to assist resource allocation and surveillance
- Traffic count data analysis in partnership with U.S. Forest Service and Natural Resources Canada
- Update and validation of the annual grasshopper hazard model
- Implement a web-based application for sharing geospatial data, using historic barberry eradication records as a model (Farm Bill, Washington State University)
- Development of new pest mapping technologies for improved risk analysis and support of PPQ Field Operations (in cooperation with Oregon State University)
Survey Support

- Provide scientific support for CAPS surveys of insects, mollusks, plant pathogens, nematodes, and weeds by developing pest datasheets and approved survey and identification/diagnostic methods
- Conduct pre-assessments for pests that were suggested by the CAPS community, the PPQ Pest Detection Management Team, or the New Pest Advisory Group for inclusion in the Prioritized Pest List
- Prepare outreach materials targeted for public and private industry as requested by PPQ Policy Management.
- Serve as an informal “help desk” for CAPS field staff. Respond to inquiries and requests related to survey and identification of CAPS targets
- Building screening capacity for plant pathogenic phytoplasmas (Farm Bill-funded, USDA ARS, Texas A&M, Clemson)
- Confirming the pathogenicity and host range of *Phytophthora ramorum* (Farm Bill-funded UC Berkeley)
- Improving extraction and identification methodologies for plant pathogenic nematodes (Farm Bill-funded, USDA ARS)
- Rapid and accurate diagnostic identification of phytoplasmas (Farm Bill-funded, University of Florida, USDA-ARS)

Mission Laboratory

Citrus Health - Farm Bill Funded Projects

- Support of Asian citrus psyllid/Huanglongbing detection and management programs
- Support of Asian citrus psyllid management programs and geographic information system development on crop production in the Lower Rio Grande Valley of Texas (University of Texas, Pan American)
- Contributing to HLB mitigation efforts in Texas via early detection of citrus greening disease in residential area (Texas A&M University Kingsville Citrus Center)
- Evaluating the biological control of Asian citrus psyllid
- Mass rearing biological control agents of ACP in Texas (University of Texas, Pan American)
- Bi-national partnership for the biological control of the ACP along the Mexico border (University of Texas, Pan American)
- Evaluating citrus root tissue as a target for early and efficient HLB detection (University of Texas A&M – Kingsville Citrus Center)
- Molecular pathway analysis for citrus greening disease (University of Texas Pan American)
- Sampling methodology and approach rates for *Brevipalpus* mites on limes entering the United States from Mexico
- Survey of *Brevipalpus* mites on commercial citrus in the Lower Rio Grande Valley
- *Brevipalpus* mite species on Rutaceous and non-Rutaceous plants in dooryards in the Lower Rio Grande Valley of Texas (University of Texas, Pan American)

Fruit Fly Program Support

- Methods development and quality control of Mexican Fruit fly SIT, rearing, eclosion and release
- Mexican fruit fly eradication support
- Fruit fly eradication support in Lower Rio Grande Valley of Texas (University of Texas, Pan American)
- Mexican fruit fly detection development and support
- Assessment, identification, and suppression of microbial contaminants in the Mexican Fruit Fly Mass Rearing Facility

Integrated Pest Management

- Operation of the CPHST Mission Arthropod Quarantine Facility
- Biological control of Harrisia cactus mealybug in Puerto Rico
- Quality control support of trapping for the Boll Weevil Eradication Program

Molecular Diagnostics

- Development of diagnostic tools for identifying species and geographic sources of intercepted *Dacinae* fruit flies
- Development of molecular diagnostic techniques for mollusks of economic importance to American agriculture
- Fruit fly molecular identification program to support diagnostics and pathway analysis
- Population genetics of exotic pest fruit flies (University of Texas-Pan American)
- Diagnostic resources to support fruit fly exclusion and eradication (USDA-ARS, Farm Bill-funded)
- Enhancement of fruit fly larvae identification and taxonomy (Florida Department of Agriculture and Consumer Services, Farm Bill-funded)
- *Helicoverpa armigera* molecular diagnostic tool testing
- Molecular identification of *Trogoderma* species (Coleoptera: Dermestidae)
- Molecular identification tools for the Asian gypsy moth (University of Texas-Pan American)
- Molecular markers for the Mexican fruit fly (University of Texas-Pan American)
- Molecular diagnostics of native nematodes and their potential as biocontrol agents against *Sirex noctilio* (Southern Illinois University)
- Pathway analysis and molecular diagnostic support for Asian longhorned beetle
Otis Laboratory

Agricultural quarantine and inspection

- Support for the Russian/Japanese/Chinese/Korean Exotic *Lymantria* exclusion program
- Molecular analysis of male gypsy moths trapped and intercepted in U.S. ports and other high risk areas
- Asian gypsy moth hybrids and developmental phenology
- Regulatory treatments for the control of hitchhiking insects in airplanes

Cooperative Agreements:

- Phytosanitary treatment of quarantined commodities in packaging using steam and vacuum
- Development of a methyl bromide treatment schedule for walnut twig beetle and thousand cankers disease
- Development of new treatment options for khapra beetle (Farm Bill-funded)
- Evaluation of irradiation as a phytosanitary treatment for khapra beetle (Farm Bill-funded)
- Evaluation of novel insecticide and insect growth regulator treatments for khapra beetle (Farm Bill-funded)
- Wood penetration and efficacy of methyl bromide alternatives on wood pathogens
- Development of phytosanitary treatments for exotic fruit flies
- Identification of infested wood packing material intercepted at U.S. ports
- Identification of port interceptions in wood packing material
- Molecular markers to distinguish Asian and North American gypsy moths
- Research on post-harvest and regulatory control treatments for European grapevine moth
- Development of control technologies and strategies for management of phytosanitary risks for invasive species affecting agriculture in the United States: light brown apple moth, European grapevine moth, spotted wing Drosophila, and Asian citrus psyllid
- Using steam and vacuum to heat treat hardwood veneer logs for export
- Survey detection and treatment evaluation on insects associated with wood packaging materials in China

Asian longhorned beetle

- Development of a visual trap for Asian longhorned beetle
- Assessment and comparison of survey methods for Asian longhorned beetle
- Chemical and visual ecology of wood-borers (also EAB and other wood borers)
- Improved rearing technology for Asian longhorned beetle
- Pesticide methods and plans for Asian longhorned beetle
- Evaluation of portable heat treatments for ALB infested firewood in Bethel, OH
- Infestation dynamics of ALB and EAB in North America
- Host plant utilization by the Asian longhorned beetle

Cooperative Agreements:

- Management techniques for ALB using insecticides and trapping efficacy for ALB and buprestid beetles
- Potential of nursery treatments for the ALB eradication program (China)
- ALB host range and preference testing and evaluation of insecticide treatments in China
- Applied ecology and behavior of the Asian longhorned beetle in Ohio

Emerald ash borer

- Improving trapping methodology for emerald ash borer
- Emerald ash borer biological control
- Characterization of EAB pupal chamber location in infested ash by diameter class – a potential method to eliminate need for further treatment of sawn wood
- Evaluation of systemic insecticides for EAB control
- Ecology of EAB

Cooperative Agreements:

- Developing ash varieties resistant to EAB and increasing the efficacy
- An integrated and comprehensive program to develop emerald ash borer resistant ash trees
- Development of transgenic North American ash trees expressing *Bacillus thuringiensis* protein for management of EAB
- Research and implementation of EAB biological control in Minnesota
- Optimization of methods for mass-rearing newly introduced parasitoids for biocontrol of emerald ash borer
- Assessment of new technologies to establish and evaluate introduced parasitoids of EAB – focus on the southern U.S.
- Nanofabrication of visual lures for the emerald ash borer (Farm Bill-funded)
- Developing-improving a rearing system for emerald ash borer
- Investigating the ecology and natural control of emerald ash borer in the Russian Far East
- Risk-based trapping for emerald ash borer
- Impact of EAB in Michigan: life-table analysis
- Evaluating impacts of natural enemies on population dynamics of the invasive emerald ash borer
- Evaluating success of the biological control of the emerald ash borer in New York State
- Distribution of emerald ash borer and its host trees in China

Sirex

- Chemical ecology of *Sirex noctilio*
- Biological control of *Sirex noctilio*
Cooperative Agreements:
- Sirex chemical ecology studies
- *Sirex noctilio* – identification of new pheromones and attractants from host tree volatiles
- Evaluation of *Deladenus siricidicola* trials

**Tree pests (general)**

Cooperative Agreements:
- Predicting the cumulative ecological and economic impacts of pests and pathogens in forests of the eastern U.S. (Farm Bill-funded)
- Species of insects attacking North American tree species planted in China
- Host range and distribution of *Lymantria xylina* in China

**CAPS/Pest Detection**
- Efficacy of adhesive products for capture and subsequent identification of Lepidoptera pests
- Evaluating the use of general buprestid survey traps for exotic buprestid species in Asia and Europe (EAB funding)
- Odor-based detection and monitoring systems for exotic pests

Cooperative Agreements:
- Accelerated program to develop behaviorally active semiochemicals for survey and control
- AGM female flight capabilities and *Dendrolimus*, buprestid, and *Monochamus* trapping
- Development of trapping techniques for the Japanese pine sawyer
- Chemical and visual ecology of exotic wood-boring insects
- Trap and lure development for exotic wood boring beetles

Farm Bill funded cooperative agreements:
- Attraction and detection of the fruit-piercing moth
- Development of a novel insect trap for detection of invasive plant pests
- Improved traps and lures for moths
- *Tuta absoluta* sex pheromones – detection and management tools
- Remote sensor-smart trap development
- Evaluation and development of survey techniques for *Lymantria dispar* and *Dendrolimus* spp.
- Developing survey tools for the hemlock caterpillar
- Chemical ecology and behavior of the polyphagous shot hole borer
- Attraction and detection of polyphagous shot hole borer *Eucosma lineata* spp.
- Development of detection tools for exotic buprestid beetles
- Enhancing survey techniques for the exotic goldspotted oak borer in southern California
- Generic pheromone blends for early detection of longhorned beetles
- Development of chemical attractants and improved trap design to facilitate detection of exotic Cerambycidae
- Behavioral study and attractant development for the citrus longhorned beetle
- Development of attractants for *Apriona germari* and *Batocera borsfieldi*
- Tree injection and other methods to improve trapping of wood boring beetles

**Specialty crop pests**
- Rearing system development for European grapevine moth
- Support for PPQ programs in California

Cooperative Agreements:
- Development of stable isotopes and new biochemical tools for identification of sterile insects and determination of pest origin
- Development of IPM and biological control strategies for management of Asian citrus psyllid (ACP) in California
- Monitoring and control of European grapevine moth, *Lobesia botrana*
- Radiation biology of European grapevine moth (Farm Bill-funded)

**European gypsy moth**
- Production of gypsy moth virus
- Rearing gypsy moths for research and outreach projects

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

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

**Accreditation and Certification of Risk Analysis Functions**
• Through audits and improvements, maintain ISO certification for the Lab’s commodity risk assessments and New Pest Advisory Group

**Outreach and Training/Capacity Building/Regulatory Curricula**
• Provide instructors for a regulatory science minor at North Carolina State University (NCSU)
• Maintain the strong cooperative relationship established between CPHST, NCSU, and other academic institutions
• Support a regulatory curriculum that provides training to students in relevant fields on key aspects of regulatory plant protection
• Host risk analysts from other countries, pairing visiting scientists with resident analysts as mentors to provide training in risk analysis methods

**Plants for Planting (Q-37) Analyses and Regulatory Overhaul**
• Provide scientific and strategic support to revise and update 7 CFR 319.37, the quarantine that regulates the import of plants for planting
• Advance the regulatory process through the development of methodologies and analyses to support the APHIS decision-making processes associated with the evaluation of pest risk prior to authorizing the entry of propagative material into the United States
• Prepare risk assessments for propagative material proposed for importation

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

**Information Systems and Biosurveillance Analysis Forecasting**
• Maintain and expand the Global Pest and Disease Database (GPDD)
• Through the NCSU/APHIS Plant Pest Forecast (NAPPFAST) System, support the predictive pest mapping needs of the Cooperative Agricultural Pest Survey (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 APHIS PPQ CAPS Program by producing pest prioritization lists using the analytic hierarchy process
• Co-lead the development of PestLens, the new biosurveillance system for PPQ

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

**Reference Management**
• Maintain and improve physical library
• Maintain and improve PRA library in Endnote
• Maintain and improve the digital library of scientific references
• Maintain and improve the EEO library
• Maintain and improve the health and fitness library
Phoenix Laboratory

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

- Investigation of wild cotton as a pink bollworm host in the Pink Bollworm Eradication area and identification of similar species trapped in wild cotton
- Preparation and shipment of insect diet to cooperators
- Implementation of method for high performance liquid chromatography as a quality control method for dietary levels of marker in diet
- Development and maintenance of bibliographies to gather and house documents regarding current research topics
- Rearing, documentation, and shipping of genetically modified PBW used as quality assurance specimens in the PBW Eradication Program
- Identification of and recommendations for the mitigation of sources of escaped moths from mass-rearing facility including the rearing and release of genetically marked, sterile moths around the rearing facility
- Comparison of current formalin egg sanitation treatment to Oxiver Five 16 and CHG in small scale and in larger scale experiments
- Preparation for small scale releases of sterile moths to respond to trap catches, post-eradication, by applying for certificate of airworthiness for an unmanned aircraft
- Insect diet research and upgrades to twin-screw extruder to meet research needs

Rangeland Grasshopper/Mormon Cricket
- Partnering with industry to develop a new pyrethroid toxicant for use as a bait to suppress grasshoppers and Mormon crickets on rangeland
- Conduct operational scale evaluation of chlorantraniliprole (Prevathon) for control of rangeland grasshoppers
- Provided recommendation for use of Prevathon from 4 fl. oz. blanket or down to 2 fl. oz. RAATs application depending on grasshopper density
- Evaluation of applications of selected domestic strains of pathogenic fungi in mini plots, for control of rangeland grasshoppers (Orthoptera: Acrididae) near Sidney, MT
- Collection, cataloging, and screening of domestic fungi pathogenic from soil samples (Utah State University)
- Continue to acquire, catalog, and make available grasshopper and Mormon cricket field research data, historical documents, and grey literature documenting the history of grasshopper and Mormon cricket control programs conducted by USDA, APHIS, PPQ
- Selecting Metarhizium strains from bio-catalog for further field evaluation of strengthened UV tolerance based on replicated laboratory trials

Biological Control Program

A full listing of the project titles and publications of CPHST scientists working on biological control can be found under the highlights and funded projects sections for the individual CPHST laboratories, with the exceptions of Amy Roda and Scott Wehman at the Miami Station and Pedro Rendón at the Guatemala Station, who are administered from the Director’s Office in Raleigh, NC.

Coordinator Cooperative Agreements
- Developing biological control and other safeguarding tools to manage invasive pests (Florida A&M University)
- Understanding the economics of biological control (Florida A&M University)

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

Miami Station
- Assessing biological control options for Harrissa cactus mealybug in Florida and Puerto Rico
- Designing methods to trap giant African snails in urban and suburban settings
- Strategies to slow the spread of Tuta absoluta (University of Panama)
- Land snail aggregation pheromones: A tool to detect and control giant African snail (UC-Riverside)
- Mitigating invasive pests in Puerto Rico: A front line initiative for rapid response (Farm Bill-funded)
- Screening traps using portable computer based microscopes (Farm Bill-funded)
Fruit Fly Program

CPHST headquarters
- CPHST Quality Assurance Survey Program in support of national fruit fly trap and lure procurement
- Fruit systems approaches and regulatory treatments using foliar sprays
- Fruit fly host compendium

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

Mexican Fruit Fly Program
- Developed egg-bubbling and handling procedures
- Determined best artificial lure to use in MFF monitoring and trapping
- Mexican Fruit Fly Sterile Insect Monitoring Model
- Methods for detecting and combatting three Morganella bacterial isolates
- Effect of loading individual trays in Worley tower system at different pupal densities
- Check protocols and standardize procedures in mass rearing facility

Hawaii and California Fruit Fly Support
- Hawaiian Pacific Basin Center for Insect Control - PBACIC
- Effects of chill duration on flight ability in sterile male Medflies
- Mark-release-capture of sterile male medflies to describe post-release dispersal.
- Field study examining the effects of pre-release exposure to methyl eugenol on the mating competitiveness of sterile males of B. dorsalis
- Distribution patterns of flies released using current aerial release protocols
- Effect of chilling and the knockdown procedure on sterile medflies
- Morphological training on identification of over 50 species of economically important Bactrocera and Dacus species in Oahu, Hawaii
- Compendium of Host Fruit Fly Information (CoHFFI) to determine field infested hosts for economically important fruit flies
- Quality control of tsl sterile males
- Systems approach work to facilitate trade movement of conditional fruit fly hosts
- Effects of ground release
- Evaluate the best density of pupae in trays and towers in mass rearing facility
- Editor for Trapping and the Detection, Control, and Regulation of Tephritid Fruit Flies (will be published in 2014)

Guatemalan Moscamed Operations Support for Fruit Fly Preventative Release and Eradication Programs
- Field evaluations of traps and lures as part of CPHST QASP to support procurement and contracting of products to use in domestic fruit fly programs
- Alternatives to replace the use of agar to feed sterile Medflies – Potential savings when implemented.
- Fabrication and testing of bait stations for use in fruit fly programs both nationally and internationally that could results in significant cost savings to program by reducing the need for ground bait sprays during outbreaks, associated fuel costs and labor.
- Evaluation of bait station efficacy as it relates to storage and shelf-life. Bait stations can be stored for at least two years.
- Evaluation of diet ingredients for Medfly production at El Pino Medfly Rearing Facility in Guatemala. Findings will reduce egg production costs.
- Improved utilization of sterile insects through development and use of a monitoring tool to calculate release densities based on sterile to fertile fly ratios
- Contributing to updated Production QC Manual for sterile insect mass rearing facilities

National Plant Protection Laboratory Accreditation Program
- Enhanced Plant Pest-Disease Analysis of the National Plant Diagnostic Network Repository.
- NPDN STAR-D Program Development and Auditor Training.
- Accreditation System for Plant Diagnostic Network and Regulatory Laboratories - Development and Deployment.
- NPDN Diagnosticians Training Sessions in Regulatory Diagnostics.
• Molecular Diagnostic Testing for Phytophthora ramorum for the 2012 USDA Regulatory Program. Cornell University, University of Florida, Michigan State University.

**Office of the Executive Director**

• Engineering mobile RNA in Carrizo rootstock to create a hypersensitive programmed cell death response in mature scions to control citrus greening. Integrated Plant Genetics, Inc.


• Stochastic modeling and the design of early detection surveys for high-risk pathogens. Rothamsted Research, UK.

• Determination if Ficus carica, Psidium guajava, Pisonia aculeata, Trichostigma octandrum and Cleome rutidosperma are alternative Hosts of Candidatus Liberibacter asiaticus, the associated bacterium of Huanglongbing. University of Florida.


• Predictive mapping to inform citrus producers of HLB/ACP risk at the Statewide, CHMA and individual block spatial levels to improve targeting of control efforts based on repeated statewide survey. Citrus Research Board of California.

• Boxwood blight mitigation strategies (USDA-ARS)

• The National Ornamentals Research Site at Dominican University of California (Dominican University of California, Oregon State University, University of California, Washington State University, USDA-ARS)

• Validating commercial formulation of Trichoderma asperellum against P. ramorum infested soil (USDA-ARS)

• Canine detection of citrus greening and canker (USDA-ARS)

• Alternative methods of eradication for potato cyst nematode (University of Idaho, Oregon State University, USDA-ARS)

• Chrysanthemum white rust mitigation and variability of isolates (Rutgers University, University of Georgia, USDA-ARS)

• Genetic detection of downy mildew and epidemiology and mitigation strategies (USDA-ARS)

• Development of molecular and antibody detection tools for Rathayibacter toxicus (Washington State University)
### Appendix B. Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACP</td>
<td>Asian citrus psyllid</td>
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<tr>
<td>AGM</td>
<td>Asian gypsy moth</td>
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<tr>
<td>AHP</td>
<td>analytical hierarchy process</td>
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<tr>
<td>ALB</td>
<td>Asian longhorned beetle</td>
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<tr>
<td>AQI</td>
<td>agricultural quarantine and inspection</td>
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<tr>
<td>APHIS</td>
<td>Animal and Plant Health Inspection Service</td>
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<tr>
<td>ARS</td>
<td>Agricultural Research Service</td>
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<tr>
<td>CANARY</td>
<td>Cellular Analysis and Notification of Antigen Risk and Yield</td>
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<tr>
<td>CAPS</td>
<td>Cooperative Agricultural Pest Survey</td>
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<tr>
<td>CBP</td>
<td>Customs and Border Protection</td>
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<tr>
<td>CDFA</td>
<td>California Department of Food and Agriculture</td>
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<tr>
<td>GLV</td>
<td>Citrus* leprosis virus*</td>
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<tr>
<td>GLV-C</td>
<td>Citrus* leprosis virus* -cytoplasmic</td>
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<tr>
<td>CVC</td>
<td>citrus variegated chlorosis</td>
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<tr>
<td>CPHST</td>
<td>Center for Plant Health Science and Technology</td>
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<tr>
<td>CFWG</td>
<td>cross functional working group</td>
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<tr>
<td>DEEP</td>
<td>Deregulation Evaluation for Established Pests</td>
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<tr>
<td>DHS</td>
<td>U.S. Department of Homeland Security</td>
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<tr>
<td>DoD</td>
<td>Department of Defense</td>
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<tr>
<td>EAB</td>
<td>emerald ash borer</td>
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<tr>
<td>EGVM</td>
<td>European grapevine moth</td>
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<tr>
<td>EPA</td>
<td>U.S. Environmental Protection Agency</td>
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<tr>
<td>FDACS</td>
<td>Florida Department of Agriculture and Consumer Services</td>
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<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
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<tr>
<td>FY</td>
<td>fiscal year</td>
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<tr>
<td>GPDD</td>
<td>Global Pest and Disease Database</td>
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<tr>
<td>GSA</td>
<td>General Services Administration</td>
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<tr>
<td>HCM</td>
<td>Harrisia cactus mealybug</td>
</tr>
<tr>
<td>HLB</td>
<td>huanglongbing (citrus greening)</td>
</tr>
<tr>
<td>IAEA</td>
<td>International Atomic Energy Association</td>
</tr>
<tr>
<td>IFA</td>
<td>imported fire ant</td>
</tr>
<tr>
<td>IPPC</td>
<td>International Plant Protection Convention</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
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<tr>
<td>ISPM</td>
<td>International Standard for Phytosanitary Measures</td>
</tr>
<tr>
<td>ITP</td>
<td>Identification Technology Program</td>
</tr>
<tr>
<td>LBAM</td>
<td>light brown apple moth</td>
</tr>
<tr>
<td>LRGV</td>
<td>Lower Rio Grande Valley</td>
</tr>
<tr>
<td>MAC</td>
<td>Multi-Agency Coordination group</td>
</tr>
<tr>
<td>Medfly</td>
<td>Mediterranean fruit fly</td>
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<tr>
<td>Mexfly</td>
<td>Mexican fruit fly</td>
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<tr>
<td>MMT</td>
<td>Medfly Molecular Tool</td>
</tr>
<tr>
<td>NAPPO</td>
<td>North American Plant Protection Organization</td>
</tr>
<tr>
<td>NCSU</td>
<td>North Carolina State University</td>
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<tr>
<td>NORS-DUC</td>
<td>National Ornamental Research Site at Dominican University of California</td>
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<tr>
<td>NPAG</td>
<td>New Pest Advisory Group</td>
</tr>
<tr>
<td>Acronym</td>
<td>Term</td>
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<td>--------------</td>
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<tr>
<td>NPDN</td>
<td>National Plant Diagnostic Network</td>
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<tr>
<td>NPPLAP</td>
<td>National Plant Protection Laboratory</td>
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<td>Accreditation Program</td>
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<tr>
<td>NSTP</td>
<td>National Scientific Technologies Program</td>
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<tr>
<td>PBW</td>
<td>pink bollworm</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PERAL</td>
<td>Plant Epidemiology and Risk Analysis</td>
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<tr>
<td>Laboratory</td>
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<td>PI</td>
<td>phytosanitary irradiation</td>
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<td>PIS</td>
<td>Plant Inspection Station</td>
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<td>PPQ</td>
<td>Plant Protection and Quarantine</td>
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<td>PRA</td>
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<td>proficiency test</td>
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<tr>
<td>QM</td>
<td>quality management</td>
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<tr>
<td>RAMBO</td>
<td>Rapid Automated Molecular Barcode</td>
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<td>Observation</td>
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<tr>
<td>RAAT</td>
<td>reduced agent-area treatment</td>
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<tr>
<td>RT-PCR</td>
<td>reverse transcription PCR</td>
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<tr>
<td>SIT</td>
<td>sterile insect technique</td>
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<tr>
<td>S&amp;T</td>
<td>Science and Technology</td>
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<tr>
<td>STAR-D</td>
<td>System for True, Accurate, and Reliable</td>
</tr>
<tr>
<td>Diagnostics</td>
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<td>SWPM</td>
<td>solid wood packing material</td>
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<tr>
<td>TPPT</td>
<td>Technical Panel of Phytosanitary Treatments</td>
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<tr>
<td>TWG</td>
<td>technical working group</td>
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<tr>
<td>UC</td>
<td>University of California</td>
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<td>USDA</td>
<td>United States Department of Agriculture</td>
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<td>United States Forest Service</td>
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<td>USGS</td>
<td>United States Geological Survey</td>
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