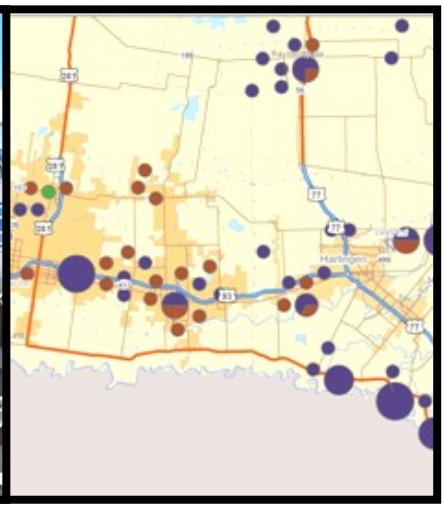


2011 Annual Report

U. S. Department of Agriculture

Animal and Plant Health Inspection Service

Plant Protection & Quarantine

Center for Plant Health Science & Technology

Mission Laboratory

FRONT COVER CREDITS

Left: Yellow colonies of *Morganella* sp. on XLD plates (Bacilio Salas) **Center:** Hugh Conway with Tim Rowland In front of airplane for fruit fly dispersal **Right:** Distribution map of mites in LRGV (Evan Braswell, Bacilio Salas, and David Bartels).

Left: Lisa Ledezma conducting molecular diagnostics (Dan Flores). **Center:** CPHST Mission Laboratory **Right:** Kristen Hopperstad examining Mexican Fruit Fly pupae (Hugh Conway).

BACK COVER CREDITS

Left: *Brevipalpus phoenicis* (Josie Salinas). **Center:** Josie Salinas inspecting limes for scarring (Danny Martinez) **Right:** Joe Martinez and Andrew Parker inspecting citrus trees (Dan Flores).

CPHST Mission Laboratory

2011 Annual Report

U. S. Department of Agriculture

Animal and Plant Health Inspection Service

Plant Protection & Quarantine

Center for Plant Health Science & Technology

Mission Laboratory

22675 N Moorefield Rd # 6414

Edinburg, TX 78541-5033

PHONE: (956) 205-7668

FAX: (956) 205-7680

CPHST WEBSITE: http://www.aphis.usda.gov/plant_health/cphst/index.shtml

MISSION LABORATORY WEBSITE: http://www.aphis.usda.gov/plant_health/cphst/

USDA Nondiscrimination Statement

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, age, disability, and where applicable, sex, marital status, familial status, parental status, religion, sexual orientation, genetic information, political beliefs, reprisal, or because all or part of an individual"s income is derived from any public assistance program. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA"s TARGET Center at (202) 720–2600 (voice and TDD).

To file a complaint of discrimination, write to USDA, Director, Office of Civil Rights, 1400 Independence Avenue, S.W. , Washington , D.C. 20250–9410 , or call (800) 795–3272 (voice) or (202) 720–6382 (TDD). USDA is an equal opportunity provider and employer.

Mention of companies or commercial products does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned. USDA neither guarantees nor warrants the standard of any product mentioned. Product names are mentioned solely to report factually on available data and to provide specific information.

Mission Laboratory
Professional Staff

Matt Ciomperlik, Ph.D. – Laboratory Director

Elena Ibanez – Laboratory Support Assistant

Norman Barr, Ph.D. – Molecular Biologist

David Bartels, Ph.D. – Entomologist

Evan Braswell, Ph.D. – Molecular Biologist

Hugh Conway, Ph.D. – Entomologist

Roxanne Farris, M.S. – Geneticist

Daniel Flores, Ph.D. – Entomologist

Raul Ruiz, Ph.D. – Geneticist

Bacilio Salas, Ph.D. – Plant Pathologist

Josie Salinas, B.S. – Biologist

Don Vacek, Ph.D. – Biologist

Table of Contents

CITRUS HEALTH RESPONSE PROGRAM SUPPORT 7

Evaluating the biological control of Asian Citrus Psyllid in the Lower Rio Grande Valley of Texas 8

Development of IPM strategies using interplantings for management of the Asian citrus psyllid 10

Area-wide management of Asian citrus psyllid in Texas 12

Validation trials of the leaf washing process for mitigation of ACP adults on mature leaves of kaffir lime, curry, and bael 14

Studies on the effectiveness of peraclean on the viability of *Elsinoe australis*, the causal pathogen of sweet orange scab (SOS) 16

MOLECULAR DIAGNOSTICS 19

Evaluation of fruit fly identification using a DNA barcode methodology 20

Genetic analysis of the Asian citrus psyllid, *Diaphorina citri*, and its parasitoid, *Tamarixia radiata* 22

Medfly molecular pathway analysis tool development 24

Molecular genetic analysis of the citrus leprosis virus vector, *Brevipalpus* (Acari: Tenuipalpidae) 26

ARTHROPOD PEST MANAGEMENT 29

Arthropod quarantine operations of a PPQ-approved high security containment facility for support of biological control and operational programs 30

Assessment, identification, and suppression of microbial contaminants in the Mexican fruit fly mass rearing facility 32

Mexican fruit fly projects: Mexican fruit fly diet development and rearing support, operations support for fruit fly preventative release and eradication program, evaluation of new fruit fly trap/ lure/ trapping agent combinations 34

Biological Control of Harrisia Cactus Mealybug (*Hypogeococcus pungens*) 36

Rehydration of frozen boll weevils for quality control measures in the Texas Boll Weevil Eradication Program 38

CPHST MISSION LABORATORY PERSONNEL 41

CITRUS HEALTH RESPONSE PROGRAM SUPPORT

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

CPHST STAFF: Daniel Flores (lead) and Matt Ciomperlik (support)
TECHNICAL SUPPORT: Joe Martinez, Andrew Parker, Eustorjio Rivas, and Jason Carlson
CHAMPION: Kris Godfrey (CDFA)
CONTACT: Daniel Flores (daniel.flores@aphis.usda.gov)
FARM BILL PROJECT: 3.205

Accomplishments

- Received, screened, & established 2 new colonies of *Tamarixia radiata* from Southeast Asia in quarantine
- Completed host-specificity testing of *T. radiata* (Pakistan) and received release permit
- Developed two methods for mass-production
- Documented baseline data thru assessments in the Lower Rio Grande Valley
- Conducted insecticidal studies of ACP and *T. radiata*

Foreign Collection of Natural Enemies

Through an interagency agreement with ARS, arrangements were made with Dr. Matt Purcell (USDA-ARS ABCL) to visit China and Singapore in FY 2011 to make collections of new strains of *Tamarixia radiata*. We provided him with packaging material, shipping permits and labels to make the shipments to our Quarantine facility. Coordination was also made with the Los Indios Port of Entry to receive and inspect packages upon arrival into the US and to notify us immediately upon the arrival of insects. Two new collections were received, screened, and established in quarantine from Southeast Asia including Yunnan and Singapore. These bring our total to seven foreign collections that may exhibit enhanced efficacy differences or climate adaptabilities. Prior collections maintained in quarantine are from (1) Lianzhou Town, Guangxi, (2) Yangjiang City, Guangdong, (3) Guangzhou, Guangdong, (4) Hong Kong,a nd (5) Multan, Pakistan.

Host Specificity Testing

In order to complete our host specificity testing, we maintained laboratory, greenhouse, and quarantined colonies of Asian citrus psyllid, non-target psyllids, multiple collections of *Tamarixia radiata*, and various species of host-plants for these purposes. In August 2011, we completed host-specificity testing of *T. radiata* from Pakistan and received a permit for field releases in Texas. The psyllid test list included *Diaphorina citri, Bactericera cockerelli, Aphalaroida pithecolobia, Leuronota maculata*, and *Heteropsylla texana* which were all tested in PPQ's Arthropod Quarantine Laboratory. The testing at the Mission Laboratory was conducted alongside the work being done by Drs. Raju Pandey and Mark Hoddle at University of California-Riverside to meet the requirements of the permitting process. Upon permit approval, the Pakistan collection was extracted from quarantine and put into mass-production. Collections from China remain in quarantine remain in quarantine and testing is 80-90% complete.

Mass Production of Natural Enemies

Methods to produce large numbers of *Tamarixia radiata* were tested and two methods were developed for mass-

production of *T. radiata*. Protocols developed for mass production of *T. radiata* using field insectary cages on mature lemon and lime trees yielded 119,502 parasitoids using 9 caged trees. Methods developed were captured on video to use as a tech transfer tool. Protocols developed for mass production using a greenhouse, yielded 90,283 parasitoids of which 20,274 were released in a local organic grove. Through further testing of the Pakistan collection, this method may prove to be an excellent tool for introducing, releasing, and establishing exotic parasitoids directly in commercial and organic groves to help suppress ACP populations.

Endemic Parasitism Levels in the Lower Rio Grande Valley

Documented baseline data of an endemic population of *T. radiata* in the Lower Rio Grande Valley was assessed on conventional and organic citrus to one day be able to compare efficacy with future releases of exotic biological control agents. A mean parasitism level of 5.4% by *T. radiata* was observed throughout May 2010 and June 2011. Differences in parasitism levels were observed according to the variety of citrus, environmental setting (commercial vs. organic) and according to timing of year.

Toxicological Studies

Laboratory studies were conducted on ACP and *T. radiata* using Danitol and Mustang, two commonly used insecticides in Texas. Results indicated that 2% of the recommended label rates of both insecticides were sufficient to kill all adults of both ACP and *T. radiata* in less than 24 hours but mortality decreases at lower concentrations. At levels less than 2% of the recommended label rates, parasitoid insects are capable of surviving, parasitizing, and completing development and may be used in an IPM approach to reduce ACP populations while introducing parasitoids.

Development of IPM Strategies Using Interplantings for Management of the Asian Citrus Psyllid

CPHST STAFF: Josie Salinas (lead)
TECHNICAL SUPPORT: Daniel Martinez, Albino B. Chavarria, and Jose M. Renteria
COLLABORATORS: David Hall and Tim Gottwald (ARS, Fort Pierce, FL)
CHAMPION: Stuart W. Kuehn, State Plant Health Director (TX)
CONTACT: Josie Salinas (elma.j.salinas@aphis.usda.gov)
FARM BILL PROJECT: 3.203

Accomplishments

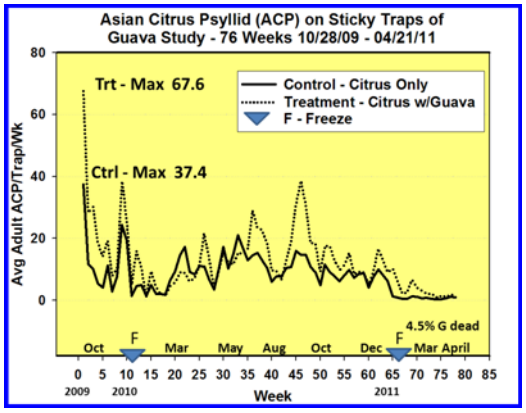
- Maintained study plots of dense interplantings of guava and citrus at USDA Mission Laboratory
- Monitored the repellent effects that volatiles released by guava leaves had on Asian citrus psyllid (ACP) populations by using sticky traps on a weekly basis
- Collaborated with Drs. Tim Gottwald & David Hall (ARS, Fort Pierce) to compare and resolve similar issues and methodology of research
- Compiled and analyzed final results of the 2-year study for using guava as a tool for managing ACP populations on citrus
- Psyllid populations over time were higher in citrus intercropped with guava than in citrus planted as a monoculture
- Submitted manuscript to scientific journal for review

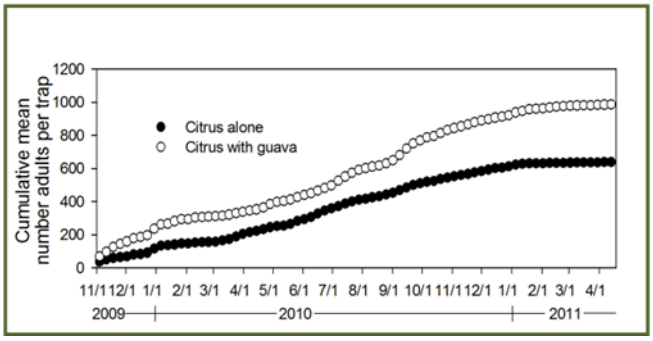
Background

Huanglongbing (HLB), also known as greening disease, is one of the most important diseases in citrus production worldwide. HLB is caused by the bacterium, *Candidatus* Liberibacter *asiaticus,* and is transmitted between citrus trees by the insect vector, *Diaphorina citri,* (Asian citrus psyllid) and/or by grafting diseased budwood onto healthy trees. No reported cases of HLB have been made to date in the citrus producing states of Arizona, California, and Texas. Since citrus trees of HLB-free states are currently at risk of being infected, the search for novel methodologies to manage HLB is critical to develop and then integrate them with more traditional management tools such as chemical vector control.

Results

Means ± SEM of 8.4 ± 0.7 and 13.0 ± 1.3 adult ACP per trap per week were observed over all 76 sample weeks in the monoculture and interplanted plots, respectively. Maximums of 37.4 and 67.6 ACP per trap per week were observed in the plots, respectively. Numbers of psyllids captured on traps were much larger than previously reported in Texas by Flores et al. (2009), although these authors studied ACP populations in mature trees. Adult ACP infestations in citrus were detected when sampling was first initiated during October 2011 in plots of citrus interplanted with guava as well as in the monoculture plot of citrus (Figure 1). The cumulative mean number of ACP per trap per week (Y) was linearly related to sample week (X). For the citrus monoculture: $Y = -182.7 + 5.7X$, slope SEM = 0.37, $r^2 = 0.88$, $F = 243$, $Pr > F = <0.0001$, 35 df; for citrus intercropped with guava: $Y = -318.0 + 9.8X$, slope SEM = 0.63, $r^2 = 0.88$, $F = 244$, $Pr > F = <0.0001$, 35 df. The slopes of the two regressions were significantly different based on 95% confidence intervals, which indicated that psyllid populations over time were higher in citrus intercropped with guava than in citrus planted alone (Figure 2).





Danny Martinez & Josie Salinas monitoring for ACP on guava.

Dense interplantings of citrus with guava.Trees were planted 2 ½ ft from each other.

Area-Wide Management of Asian Citrus Psyllid in Texas

CPHST STAFF: David Bartels (lead), Matt Ciomperlik (support), and Lisa Kennaway (GIS analysis support)
COLLABORATORS: Mamoudou Sétamou and John da Graça, TAMUK – Citrus Center
TECHNICAL SUPPORT: Jason Carlson and Oscar Pizano
CHAMPION: Stuart Kuehn, SPHD for Texas
CONTACT: David Bartels (david.w.bartels@aphis.usda.gov)
FARM BILL PROJECT: 3.207

Accomplishments

- Completed the data collection of visual observations and trapping of Asian citrus psyllid in the 4700 acres of the area-wide management pilot project
- Began the data analysis and manuscript preparation with Dr. Setamou
- Completed the first phase of geospatial analysis of the visual observation data to determine the effect of observation spacing on the estimation of ACP populations
- Developed an analysis of ACP trapping data in California to support the psyllid suppression effort

Geospatial Analysis of ACP Observation Data

The Area-Wide Management Pilot Project utilizes a uniform sticky trap grid and direct sampling observations within individual orchard blocks. The dataset contains bi-weekly observations from February 2009 to July 2011. Together, the CPHST Mission and Fort Collins Labs are using geospatial technology to manage and analyze the comprehensive dataset, with the primary goal being to identify a sampling grid that provides an accurate view of the overall ACP population, while also using fiscal resources wisely. Three grid subsets were evaluated to determine the most appropriate trap spacing for observing ACP (Figs 1 & 2). This evaluation identified several patterns in the data. First, in most cases, the half-mile or trap tree only grid will overestimate ACP populations for adult, nymphs, and percent infested variables compared with the full quarter mile grid. Second, in most cases, the half-mile and trap tree only grid will underestimate ACP egg populations. Review on this data is ongoing, and in 2012, full validation is planned. The goal of determining the best spacing for ACP observations will be extremely useful for achieving optimal balance between available fiscal resources and appropriate information required to protect orchards from ACP infestation and HLB disease. In addition, the overall interpolation analysis and zonal review will be expanded to the full set of observation and sticky trap data.

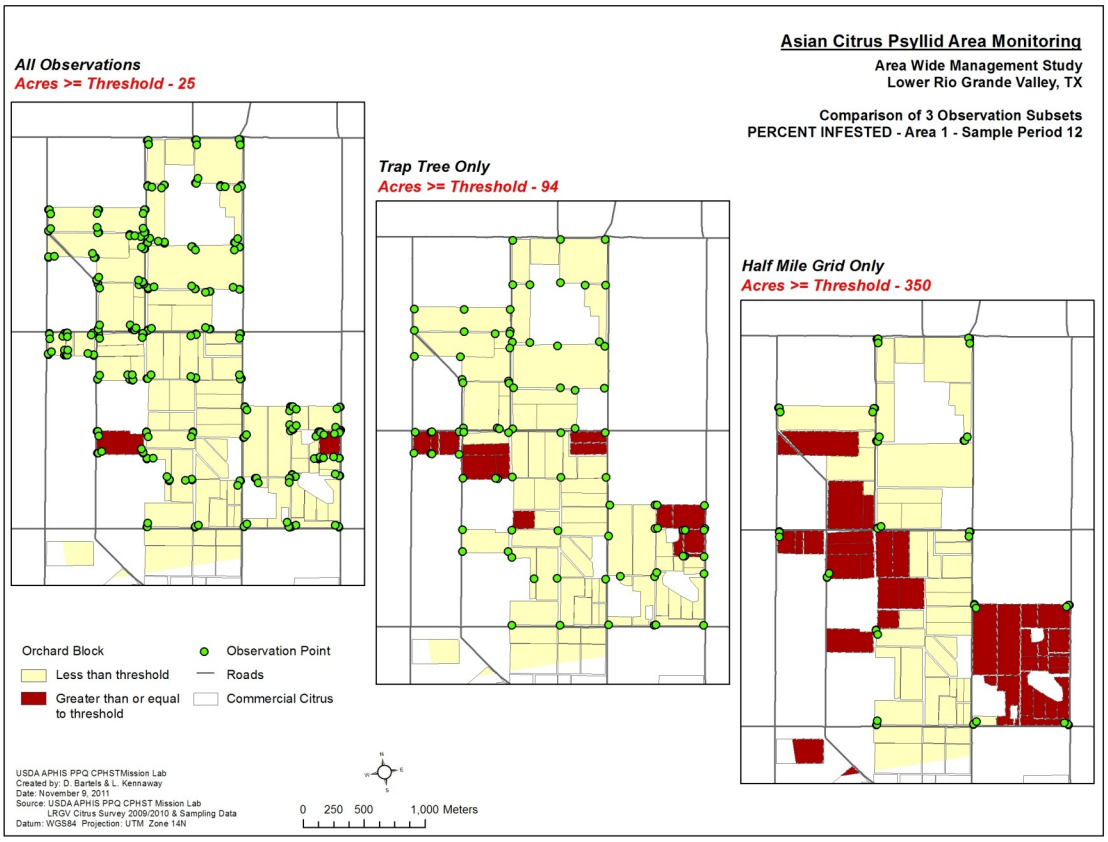


Figure 1. Map representation of the number of orchard block exceeding an adult ACP threshold based on 3 different levels of sampling.

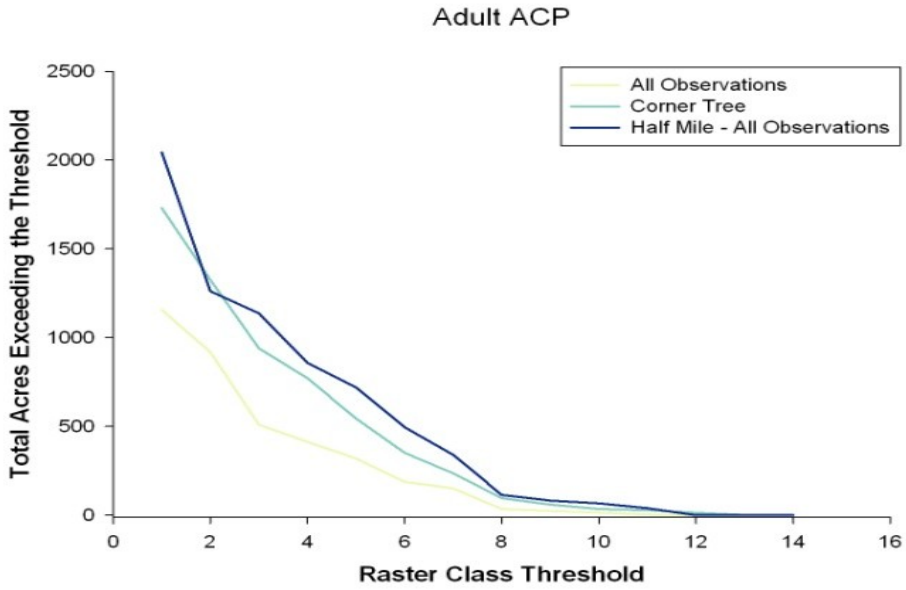


Figure 2. Graphical representation of the number of acres exceeding all thresholds for the 3 different levels of sampling of adult ACP.

Validation Trials of the Leaf Washing Process for Mitigation of ACP Adults on Mature Leaves of Kaffir Lime, Curry, and Bael

CPHST STAFF: David Bartels (lead) and Matt Ciomperlik (support)
TECHNICAL SUPPORT: Jason Carlson and Johnny Rodriquez
CHAMPION: Michael Hennessey, NSPL
CONTACT: David Bartels (david.w.bartels@aphis.usda.gov)
FARM BILL PROJECT: 3.207

Accomplishments

- Validated and demonstrated the efficacy of a leaf washing process to remove Asian citrus psyllid adults from edible citrus leaves

Validation of Leaf Washing Process

The spread of Asian citrus psyllid (ACP) into uninfested regions through citrus leaf movement is possible because leaf material has been shown to be a host for adult ACP. ACP quarantines are now in place in many areas of the U.S.A. and growers of edible leaves, such as kaffir lime, curry, and bael which are known hosts of ACP, have been impacted by these quarantines. Currently, the only accepted method to move fresh leaves out of the quarantined areas is by fumigating the leaves with methyl bromide.

Our objective was to validate the effectiveness of removing ACP adults from mature citrus leaves using a washing process. A series of studies were conducted to test; (1) the efficacy of commercially available fruit/vegetable washes to remove ACP from leaves and possible mortality effects, (2) a complete washing process that included tumbling, washing and rinsing of mature kaffir lime leaves inoculated with ACP to determine removal levels, (3) the tumbling step at two different temperatures to determine the effect on ACP removal, (4) determine the longevity of ACP at cold storage temperature, (5) effect of short term refrigerator and freezer exposure, and (6) establish any phytotoxic effects of commercially available washes on citrus leaves. Results indicate that: (1) the fruit and vegetable washes Environné, Rebel Green, and Veggie Wash had the highest removal rates on ACP, along with high mortality due to drowning in wash water (Figure 1), (2) the complete process demonstrated 99.8% removal of ACP adults and 100% removal of ACP nymphs from kaffir lime leaves (Table 1), (3) temperature did not affect removal rates of ACP adults at the tumbling step with over 99% removal demonstrated at both temperatures, (4) 82.4% nymphs were alive when only flush was present, and 89.6% of nymphs were alive when both flush and mature kaffir lime leaves was present and 79.0% of adults were alive with flush only, and 95.0% were still alive when flush and mature kaffir lime leaves was present, (5) all nymphs and adults were dead at one hour of exposure at -10˚C, but 98.9% of nymphs and 99.9% of adults were still alive after five hours of exposure to 5˚C, and (6) none of the vegetable/fruit washes produced any signs or symptoms of phytotoxicity.

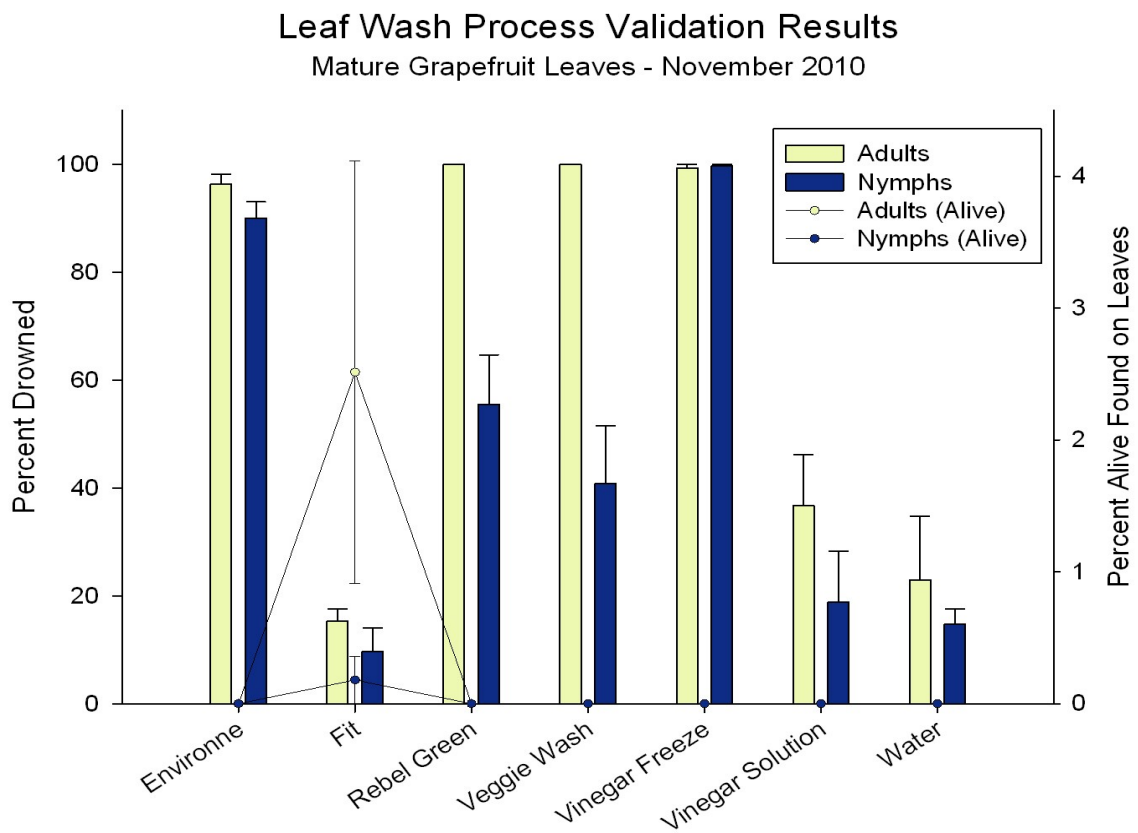


Figure1. Percent drowned of recovered ACP adults and nymphs (left axis and bars) and percentage found on leaves following the leaf washing process (right axis and lines) conducted at the Mission Laboratory, Edinburg, TX. November, 2010.

			Complete Process (25°C)		Shaking Only (25°C)		Shaking Only (5°C)	
ACP Stage	**Replicates**	**Process Step**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**
Adults	5		ACP Recovered		ACP Recovered		ACP Recovered	
		Shaking	504.0 ± 13.86	91.6 ± 2.52	413.4 ± 8.73	75.2 ± 1.59	428.2 ± 9.12	77.8 ± 1.66
		Washing	2.4 ± 1.60	0.4 ± 0.29				
		Rinsing	7.4 ± 2.06	1.4 ± 0.38				
		% Recovered		**93.4 ± 2.56**		**75.2 ± 1.59**		**77.8 ± 1.66**
		Final Inspection	1.6 ± 1.17		8.2 ± 1.11		2.8 ± 0.66	
		% Removed		**99.8 ± 0.14**		**99.1 ± 0.21**		**99.6 ± 0.06**
Nymphs	2							
		Shaking	87.0 ± 76.00	15.61 ± 13.44				
		Washing	23.5 ± 22.50	4.2 ± 4.00				
		Rinsing	7.5 ± 7.50	1.3 ± 1.34				
		% Recovered		**21.1 ± 18.78**				
		Final Inspection	0.00					
		% Removed		**100.00**				

Table 1. Mean ± SEM number and percentage recovered of ACP adults and nymphs during different stages and shaking temperatures of the leaf washing process conducted at the Mission Laboratory, Edinburg, TX. January 2011.

Studies on the Effectiveness of Peraclean on the Viability of *Elsinoe australis*, the Causal Agent of Sweet Orange Scab (SOS)

CPHST STAFF: Bacilio Salas (lead), Evan Braswell (support)
TECHNICAL SUPPORT: Armando Loya
CHAMPION: Stuart Kuehn (SPHD-TX)
CONTACT: Bacilio Salas (bacilio.salas@aphis.usda.gov)
FARM BILL PROJECT: 3.219

Accomplishment

- Documented baseline data for the effectiveness of Peraclean®5 on the viability of *Elsinoe australis*, for the citrus organic packing houses.

The discovery of the exotic citrus disease sweet orange scab (SOS) caused by *E. australis* in 2010, has caused unknown economic losses due to quarantine regulations adopted soon after its discovery. The objective of this study was to determine if *E. australis* can survive the washing, Peraclean treatment, drying, and waxing procedures at the citrus organic packing house plants.

To manage SOS, almost a simultaneous fruit washing and treatment with Peraclean®5 at the rate of 1.5% for 1-4 minutes, and exposure to 104°F during waxing are included at the organic packing house plant in Mission-TX. These treatments were evaluated under laboratory and packing house conditions in 2011. Naturally infected fruits by *E. australis* were collected by people familiar with SOS symptoms (Fig.1) from groves under organic management.

Once fruits were subjected to the corresponding treatment, *E. australis* was isolated on Whiteside's culture medium by scrapping minute tissue pieces from SOS lesions. Culture plates were incubated in darkness at 26°C for 7-10 days. Suspected *Elsinoe* colonies (Fig. 2) were transferred and identified by polymerase chain reaction (PCR).

Laboratory experiments showed that treating grapefruit for 4 minutes with Peraclean®5 at the rate of 1.5% completely inhibited the recovery of *E. australis* colonies on culture medium, whereas, *E. australis* colonies were recovered from control fruits and Peraclean®5 treated fruits for 1 minute (Fig. 3). The exposure of fruits to 104°F for 4 minutes did not have any significant effect on the viability of *E. australis*-like (data not shown). There was a significant effect of the length of time of washing on the recovery of *E. australis*-like colonies. The pathogen was recovered more often from hand washed fruits for 1 minute than from fruits washed for 3 minutes.

In organic packing house studies, washing and treating grapefruit with 1% Peraclean®5 for 2 minutes and 28 seconds reduced the recovery of *E. australis* on culture medium by 50%, whereas, in oranges the recovery was reduced by 72% after washing and treatment with Peraclean®5 at 1% for 1 minute and 42 seconds.

Fig. 1. Grapefruit with SOS symptoms.

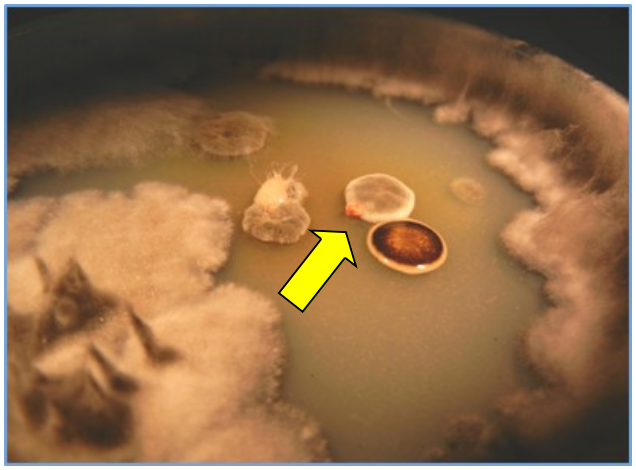


Fig. 2. Suspected *E. australis* colony on Whiteside's medium.

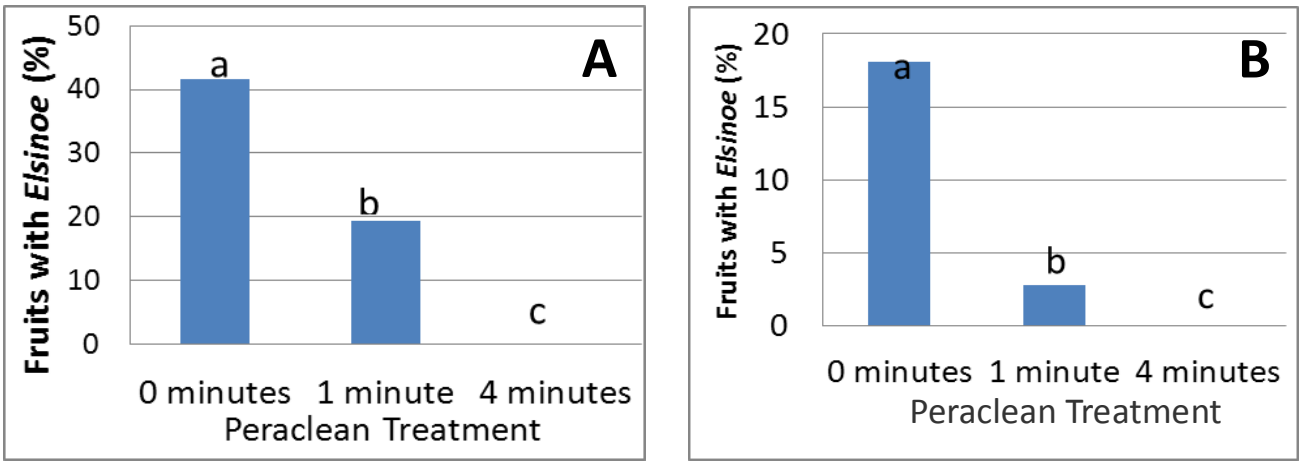


Fig. 3. Effect of 1.5% Peraclean®5 treatment on the viability of *E. australis* on grapefruit. A) Pressure spray washed fruits. B) Hand washed fruits. Means followed by the same letter do not differ significantly at P0.05 according to LSD test.

MOLECULAR DIAGNOSTICS

Evaluation of Fruit Fly Identification Using a DNA Barcode Methodology

CPHST STAFF: Norman B. Barr and Roxanne E. Farris
Technical Support: Terrance Todd, Juan Daniel Vasquez, and Lisa Ledezma
CHAMPIONS: Wayne Burnett (EDP Director of Fruit Fly Programs)
CONTACT: Norman Barr
norman.b.barr@aphis.usda.gov, 956-205-7658
NATIONAL INITIATIVE PROJECT: 179-817-P-210

Accomplishments

- Development of DNA sample repositories from expertly identified insects
- Generation of DNA barcode libraries for economic pests in the genera *Anastrepha* and *Ceratitis*
- Evaluation of utility of DNA barcode for identification of pest species
- Evaluation of DNA barcode implementation using existing methods and facilities

Fruit Fly Identification

There are over 5,000 species of fruit flies in the Family Tephritidae. It is estimated that nearly 8% of these flies are of economic importance and several are ranked among the world's most destructive agricultural pests. Species like the Oriental fruit fly and Mexican fruit fly (Figure 1) are targets of expensive exclusion and eradication programs.

Although fruit fly species can appear very similar based on body and wing structure, they can still have different diets, behaviors, and tolerances to climate and habitat. These differences affect how PPQ conducts trapping and eradication programs in response to fly detections and outbreaks.

Accurate identification of fruit fly species is important to ensure that best practices for eradication are being implemented. In most situations, fruit flies can be identified to species by trained entomologists. Unfortunately, when flies are detected as eggs, larvae, or pupae, those traditional methods of identification are not often reliable. In such situations, insect DNA can be used instead to diagnose the pest.

Over the past decade, scientists have proposed that it is possible to identify all insect pests using a single methodology called DNA barcoding. In theory, by comparing differences in a gene that is present in all animals, it may be possible to identify all fruit flies! If possible, this DNA barcode approach would reduce the time and costs associated with developing unique protocols for numerous pest species. Few studies have evaluated the utility of this method for fruit fly identification.

Performance of DNA Barcodes for Fruit Fly Pests

In 2011, scientists from PPQ-CPHST, USDA-ARS, Penn State University, Estadual University of Santa Cruz in Brazil, Royal Museum for Central Africa in Belgium, and California Department of Food and Agriculture collaborated on a project to generate DNA barcode resources (called libraries) for two economically important groups of fruit flies: *Anastrepha* and *Ceratitis* (Figure 2). Over 300 exotic species of fruit fly belong to these two groups. These species represent nearly 20% of the known pests in the entire family. The genus *Anastrepha* includes many of the New World pests such as the Mexican fruit fly, the South American fruit fly, and the West Indian fruit fly. The genus *Ceratitis* includes the highly invasive Mediterranean fruit fly.

Figure 1. The Mexican fruit fly, *Anastrepha ludens* (image from BugGUIDE.net)

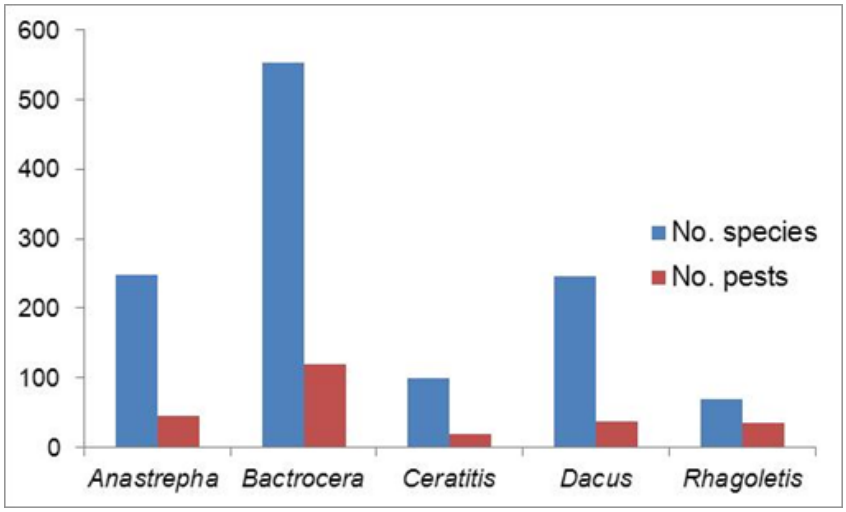


Figure 2. The number of species and pests within the five most economically important groups of fruit flies

Based on examination of DNA barcode libraries comprising over 850 DNA records and nearly 120 species, the method works well for many non-pest species and some pests. However, it was not possible to diagnose all the pests because the DNA barcodes of some species were too similar. Fruit fly species that are very closely related and cannot be reliably diagnosed using outward appearance alone are often labeled as “species complexes”. These complexes are problematic for traditional methods of diagnosis used by USDA entomologists. Our results demonstrate that these complexes are problematic for the DNA barcode technology as well.

The ability to analyze numerous fly species using a single protocol was also tested. As expected, we found that a single protocol worked well for the majority of species. However, to generate results for all species, slight modifications were required. Analysis of 547 port-intercepted larvae from passenger baggage was also conducted to evaluate protocol success. Over 97% of the tested samples generated usable DNA showing that the method is robust. Therefore, the DNA of larvae collected by inspectors and screened by entomologists should be adequate for future genetic analysis. Despite robustness in the method, it is not yet inexpensive or rapid.

Although the DNA barcode method alone cannot diagnose all important fruit fly species, it can be used in conjunction with other sources of information to make important decisions. Based on our work, however, DNA barcodes will not be a panacea for fruit fly identification. The Mission Lab is using its DNA repository generated in 2011 to investigate additional methods for fruit fly species identification and to work with international researchers to answer important questions concerning species complexes. The results of the 2011 fruit fly research project have been published in the Annals of the Entomological Society of America or released in CPHST reports.

Genetic Analysis of the Asian Citrus Psyllid, *Diaphorina citri*, and its parasitoid, *Tamarixia radiata*

CPHST STAFF: Evan Braswell
TECHNICAL SUPPORT: Terrance Todd and Rosie De Leon
CHAMPION: Patrick Gomes (PPQ)
CONTACT: Evan Braswell (evan.braswell@aphis.usda.gov)
FARM BILL PROJECT: 3.212

Accomplishments

- Confirmed the identity of parasitoid offspring reared during host-range testing of *Tamarixia radiata*, a biocontrol agent of Asian Citrus Psyllid;
- Documented mitochondrial genetic diversity in among populations of *T. radiata*, a biocontrol agent of ACP;
- Documented mitochondrial genetic diversity in among populations of *Diaphorina citri*, the vector of HLB;
- Identified useful microsatellite markers in *D. citri* and established multiplex reaction panels to evaluate these markers efficiently;
- Collecting microsatellite data for worldwide collections of *D. citri*.

Background

Citrus greening disease, also known as "huanglongbing" (HLB) disease, is one of the most serious diseases of citrus. With 328,520 bearing hectares of commercial citrus located in Florida, Texas, Arizona and California and an annual value of $2.98 billion at risk, HLB poses a serious threat to the U.S. citrus industry.

The causative agent of the disease, the bacterium *Candidatus* Liberibacter asiaticus, is vectored through feeding activity of the Asian Citrus Psyllid (ACP). This widespread psyllid has invaded citrus growing areas of the United States over the past decade. First found in Florida in 1998, the psyllid was discovered in Puerto Rico and Texas only three years later. Subsequently, ACP has been found to be widespread having been documented in South Carolina, Georgia, Alabama, Louisiana, Texas, Arizona, and California.

HLB disease was first detected in the United States in 2005 in southern Florida and subsequently in South Carolina, Georgia, Louisiana, Puerto Rico, the Virgin Islands and most recently, Texas. Despite widespread presence of the vector, ACP, the disease has not yet been detected in Alabama, Arizona, or California. Understanding the origins, dispersal patterns, and fate of introduced populations of these two species will aid our understanding of the predicted responses of both pest and parasitoid to each other.

Worldwide Genetic Diversity

Analysis of over 600 psyllids from around the world has identified 10 unique mitochondrial haplotypes (Fig 1) and their distribution (Fig 2) around the world. Interestingly, we identified a novel haplotype in the Caribbean that was present in a small number of recently collected Texas ACP. As this haplotype is missing from older collections in Texas, a recent invasion of Texas seems likely. We are investigating three routes of invasion: West through Florida, North through Texas ports and North through Mexico. Florida lacks this haplotype in both recent and older samples and samples from Mexico are being collected and tested currently. Due to the relatively poor resolution of mitochondrial DNA in ACP, we are developing microsatellite markers to achieve greater resolution for pathway analysis.

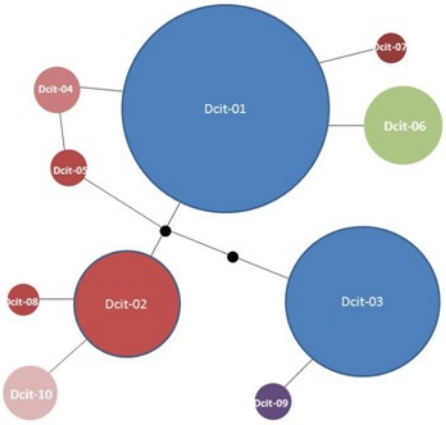


Fig. 1. Phylogenetic network depicting relationships among unique ACP sequences.

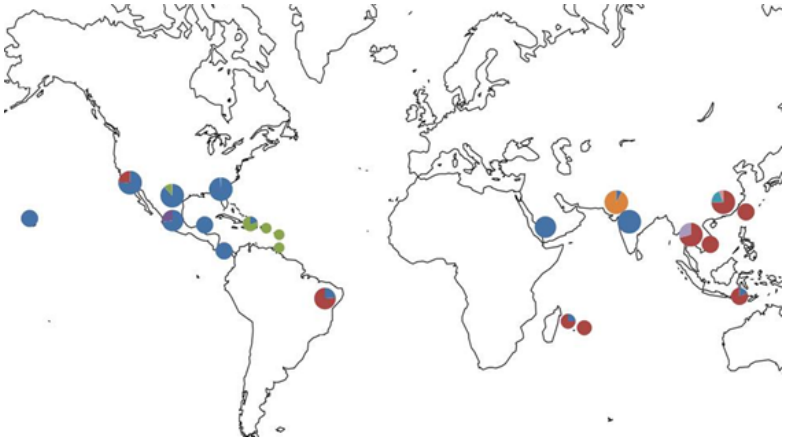


Fig. 2. Distribution and abundance of ACP mitochondrial haplotypes. Colored pie pieces indicate haplotype frequency.

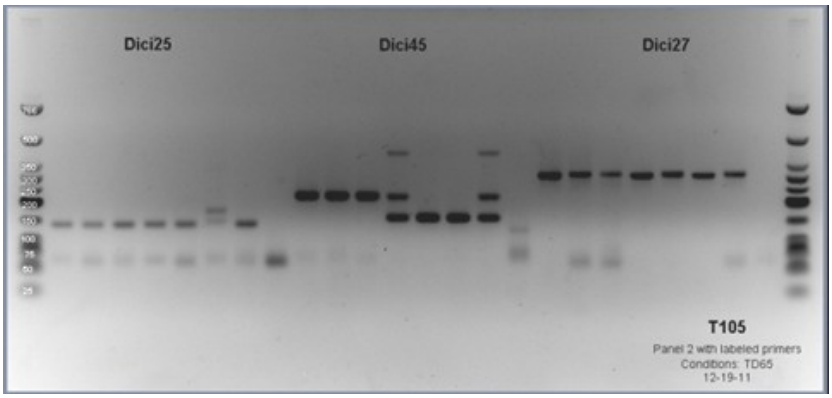


Fig. 3. Agarose electrophoresis gel of individual PCRs of three microsatellite markers: Dici25, Dici 45, and Dici27.

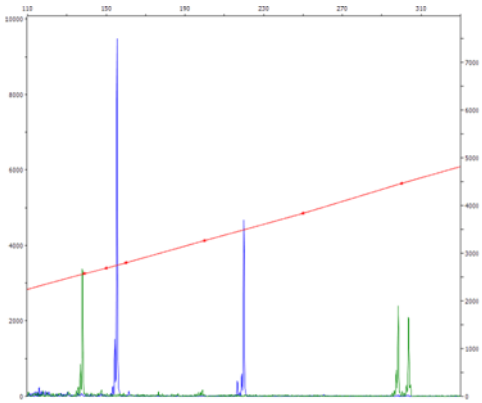


Fig. 4. Electropherogram depicting simultaneous analysis of three microsatellite markers utilizing size and dye type to distinguish Dici25, Dici45, and Dici27.

Microsatellite Development

We have developed microsatellite markers for ACP and *T. radiata*. *T. radiata* has exhibited strikingly little genetic variation and we are in the process of determining whether this lack of variation is widespread. For ACP, we optimized multi-plex microsatellite reactions allowing data from 16 genomic locations per individual while using the resources for only 6 reactions, a cost savings of 63% (Figs 3 and 4). We are collecting microsatellite data from populations of ACP around the world to identify pathways of spread and to identify origins of invasive populations.

Medfly Molecular Pathway Analysis Tool Development

CPHST STAFF: Raul Ruiz-Arce (lead); Norman B. Barr (support)
Technical Support: Terrance Todd and Fritzie Into
CHAMPIONS: Phil Garcia (WR Regional Director)
CONTACT: Raul Ruiz (raul.a.ruiz@aphis.usda.gov 956-205-7675)

Accomplishments

- Increased the APHIS Mediterranean fruit fly repository collection
- Successfully sequenced over 1800 flies collected world wide
- Recovered over 200 unique sequence types, many that have geographic association
- Evaluation of using combined DNA regions suggests improved resolution as compared to previously used methods

The Mediterranean fruit fly, *Ceratitis capitata,* is a pest that feeds and develops on over 250 different plant species. It is capable of invading many habitats and is currently established in many agricultural areas around the world (Fig. 1). Considered among the most economically important pests, numerous Medfly captures have been recovered on US soil over the last few decades. These include multiple finds in Florida and California. The costs for eradication and management of established Medfly are enormous and determining the route of introduction, or pathway analysis, is an important component in pest exclusion efforts. Pathway analysis provides valuable information to help programs develop the most effective strategy for pest mitigation.

Methods currently used by PPQ to identify the Mediterranean fruit fly cannot determine the geographic source of an intercepted fly. Several techniques using DNA have been developed over the years to observe differences in fruit fly populations. These studies, however, included flies collected from only a few geographic locations or used techniques that can only provide crude resolution. Our CPHST lab is working to examine these technologies, expand the number and range of geographic samples, and develop the methods into an effective pathway analysis tool appropriate for PPQ needs.

As one of the essential goals of this project, we initially set out to increase our reference fly collections. Capturing the genetic diversity from the broadest possible geographic collection of Medfly is vital to developing an effective pathway analysis method. It is important that the collections represent the most recent and accurate distribution of genetic types for this and many other pest species. In order to accomplish this goal, we collaborated with many researchers both here in the US and worldwide. As a result, over the last few years we received over 15,000 Medflies from over 100 geographic sites, some representing historical collections of captures made on US soil. A portion of the samples were used in the development of molecular tools that will hopefully enhance our ability to accurately determine the source of a capture.

For the past 10 years, the predominant method of medfly pathway analysis relied on a single region of DNA located in the insect's mitochondrial genome. This "single-locus" approach provided useful information but was not capable of fine-scale resolution. At the CPHST Mission Lab, the molecular tool we're developing examines the DNA from numerous genetic regions within a fly. This "multi-locus" tool combines DNA sequence information from a fly's mitochondrial genome with fragment information from microsatellite DNA of the fly's nuclear genome in order to arrive at a more informative pathway analysis result. This is the most ambitious undertaking thus far for any Medfly population study.

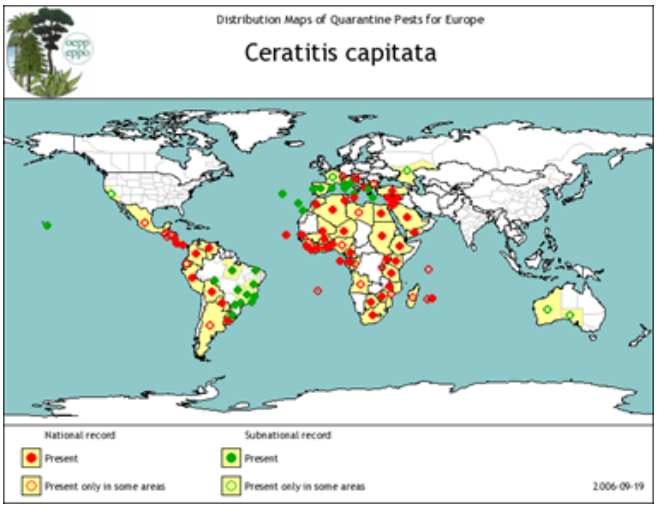


Figure 1. Geographic distribution of *C. capitata* collections.

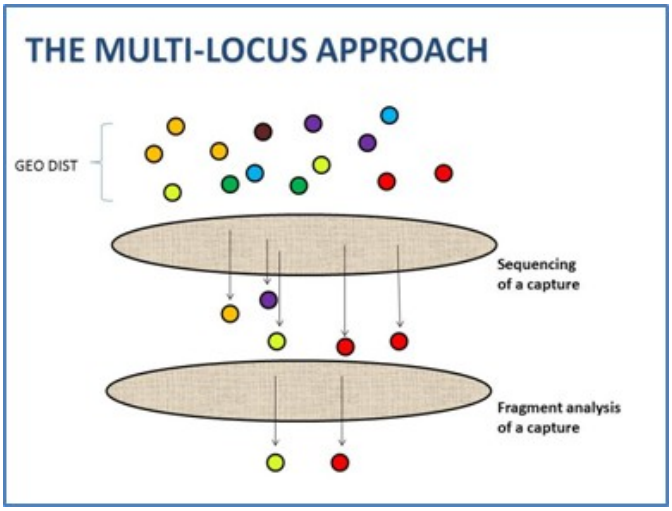


Figure 2. Diagram illustrating the use of both sequencing and fragment analysis methods for revealing the likely source of a Medfly introduction.

Results

We successfully sequenced approximately 1800 flies from over 50 Western and 60 Eastern Hemisphere collection sites. Fragment analysis of microsatellite DNA is ongoing. So far, our preliminary results have revealed over 200 unique mitochondrial sequence types, many that have geographic association. Much more remains to be done, but we have seen that our analyses suggest that when used in tandem, the mitochondrial methods identify the pathway to a regional-level (country/region) and microsatellite DNAs resolve to an even finer geographic scale. What this means is that the use of the combined DNAs (mitochondrial and microsatellite) has increased our power of resolution greatly as compared to sequencing alone as well as compared to some previously used methods.

The process for examining a fly capture is fairly straight forward but analysis and interpretation of results require staff with training in statistical genetics. First, the mitochondrial DNA sequence of the capture is compared to a reference database of over 200 unique mitochondrial types. This allows us to quickly exclude many potential geographic sources using the mitochondrial sequencing method. This step has …. A filtering effect, if you will (Fig. 2). We then analyze the fly's microsatellite DNA, comparing the microsatellite DNA results to those from geographic sources not excluded by the sequencing analysis. Consequently, we narrow our search to a finer geographic scale using the microsatellite DNA. This then reveals a more accurate list of geographic sources that represent the likely source of the introduction.

We anticipate data collection and statistical analysis of the tool to be complete in FY12. Based on in-depth analysis of the completed resource, we will develop recommendations on likely pathways for past captures and identify ways to improve the resource for future PPQ needs.

Molecular Genetic Analysis of the Citrus Leprosis Virus Vector, *Brevipalpus* (Acari: Tenuipalpidae)

CPHST STAFF: Evan Braswell (lead), Bacilio Salas (support), David Bartels (support)
TECHNICAL SUPPORT: Kristen Hopperstad, Armando Loya, and Chris Lukaszewski
CHAMPION: Stuart Kuehn (SPHD-Texas)
CONTACT: Evan Braswell (evan.braswell@aphis.usda.gov)
FARM BILL PROJECT: 3.206

Accomplishments

- Developed molecular tools to distinguish *Brevipalpus* mites from other citrus-feeding mites;
- Identified the species of *Brevipalpus* mites present in the Rio Grande valley;
- Assessed the distribution and abundance of *Brevipalpus* species in the Rio Grande valley;
- Assessed the host range of the *Brevipalpus* species in the Rio Grande valley.

Background

Citrus leprosis virus (CiLV) nearly destroyed the citrus industry in Florida between 1906 and 1925 (Childers et al., 2003). Currently, the disease is no longer present in Florida, and has not been detected anywhere else in the U.S. CiLV is present in Argentina, Brazil, Bolivia, Colombia, Venezuela, and in most Central American countries. Its recent finding in southern Mexico has created fears that the disease could eventually reach the U.S. and cause serious economic losses to the $10.5 billion citrus industries in Florida, California, and Texas.

CiLV is vectored by the false spider mites, or flat mites, *Brevipalpus phoenicis*, *B. californicus*, and *B. obovatus*. Mites of this genus are widely distributed in citrus producing areas, increasing the possibility of establishment if CiLV were introduced. Because one of the primary missions of APHIS is to safeguard U.S. agriculture from risks associated with entry, establishment, and spread of exotic pests and pathogens, it is very important to survey for both the CiLV and its vector in all citrus producing states.

Safeguarding American citrus from CiLV will depend on reliable methods of identification of the *Brevipalpus* species and detection of the virus in the mites. Morphological identification of this mite species may be difficult due to its minute size and similarity to other closely related mites. Molecular methods provide a means to accurately identify species and can potentially be useful for population discrimination.

Identification

We sampled 285 trees from 11 varieties of citrus (Fig. 1). From these samples we acquired 245 mites. DNA sequences from these mites showed pairwise identity to GenBank sequences varying from 100% to 84%. Until morphological identifications could be made, mites were assigned names corresponding to their GenBank closest match, placing the mites into three families across two orders. The weakest similarity was our “Suidasia-like” sequence which showed 84% identity to its closest match Suidasia medanensis. Phylogenetic analysis supported these temporary classifications by grouping “species” into expected relationships. If these groupings are accurate, it suggests that *B. phoenicis* is basal to *B. obovatus* and *B. californicus*, while *B. obovatus* is paraphyletic (Fig.2).

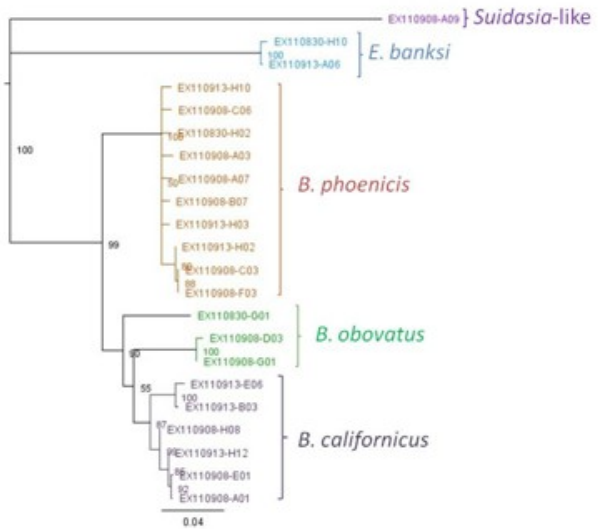


Fig. 1. Phylogenetic tree depicting relationships among unique mite mitochondrial DNA sequences.

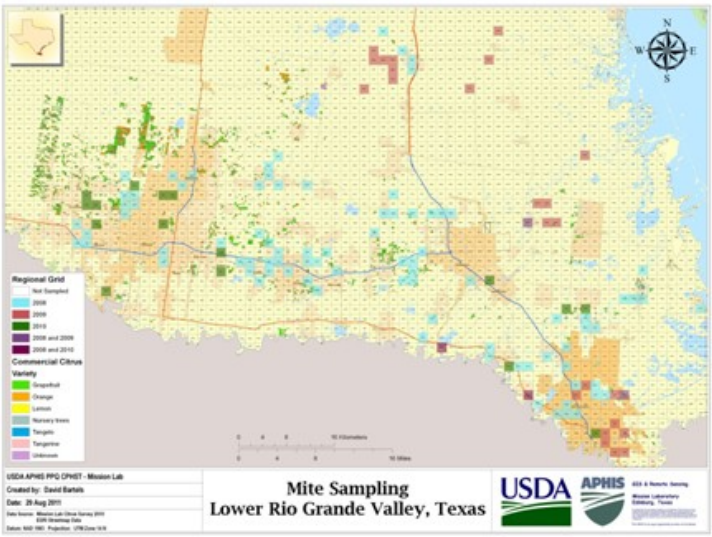


Fig. 2. Map of the lower Rio Grande Valley in Texas indicating sampling grid, predominant crop variety and year each site was sampled.

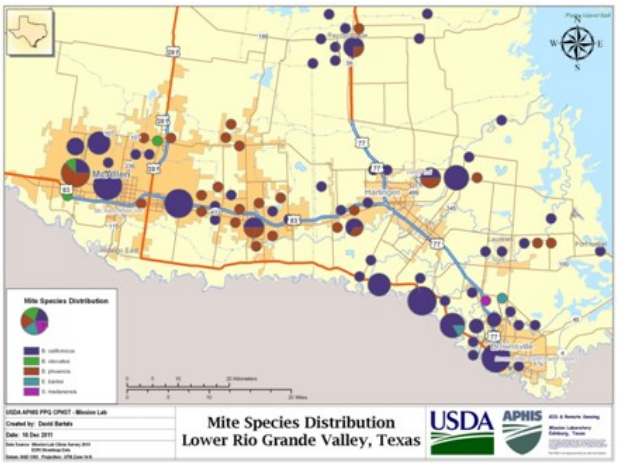


Fig. 3. Distribution and abundance of mites. Colored pie pieces indicate frequency and circle size indicates relative population size.

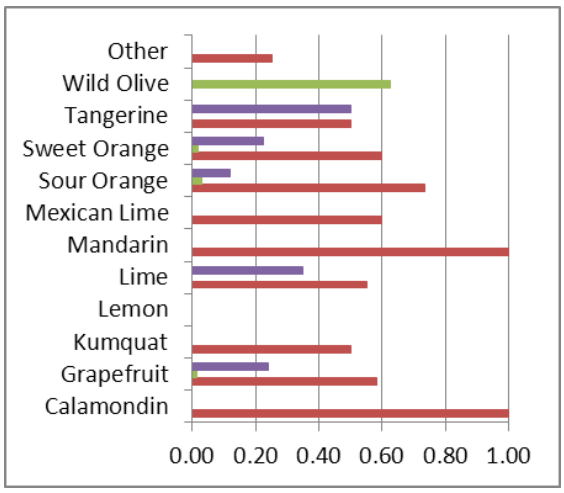


Fig. 4. Number of Brevipalpus mites per tree.

Identification

Of our 245 mites, *B. californicus* was the most common accounting for 68% followed by *B. phoenicis* at 25% while *B. obovatus* only accounted for 4%.

B. californicus and *B. phoenicis* are distributed across the valley, however, *B. phoenicis* appears to be lacking from the southern most collection sites (Fig. 3). The rarest member of the genus *B. obovatus* appears to be limited to the area surrounding McAllen (Fig. 3). Whether this restricted distribution is due to limited sample size, host restrictions (see below) or limited spread is unknown (Figs. 3 and 4).

ARTHROPOD PEST MANAGEMENT

Arthropod Quarantine Operations of a PPQ-Approved High Security Containment Facility for Support of Biological Control and Operational Programs

CPHST STAFF: Daniel Flores (lead), Albino Chavarria (support), and Matt Ciomperlik (support)
CHAMPION: Stuart Kuehn (SPHD-TX)
CONTACT: Daniel Flores (daniel.flores@aphis.usda.gov)
PROJECT: M4C01

Accomplishments

- Supported APHIS Asian citrus psyllid biological control project
- Supported ARS *Arundo donax* biological control project
- Supported Mexican fruit fly wild colony maintenance

The facility consists of a receiving lab, an identification lab, three laboratories, twelve walk-in environmental growth chambers, three reach-in environmental growth chambers, a headhouse/ potting area adjacent to five separately controlled greenhouses, a pot and clothing washing area, storage area and lunchroom. One pass-through autoclave is used for waste sterilization. Movement of personnel in and out of the facility is channeled via pre– and post-entry vestibules through separate men's and women's restrooms/ dressing rooms. Entry of biological materials into quarantine and exit of permitted materials out of quarantine is conducted through a secured-access pass-through chamber. The Arthropod Quarantine facility continues to support several PPQ programs through secure receipt, handling, rearing, and permitted release of numerous biological control agents. During FY 2010, the facility received multiple shipments of natural enemies in support of the Asian citrus psyllid, Russian knapweed, and giant cane (*Arundo*) biological control programs and maintained a wild colony of Mexfly in support of the eradication program. A list of organisms with active permits is shown in Table 1. Documentation of the movement of all personnel, organisms, and materials is also collected. A summary of archived data is shown in Table 2. Projects conducting studies in Quarantine follow:

Asian Citrus Psyllid (*Diaphorina citri*) - The Asian citrus psyllid, *Diaphorina citri* (Kuwayama) (Homoptera: Psyllidae), biological control project received two foreign shipments of the parasitoid, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) from Yunnan, China and Singapore bringing the number of foreign collections to seven. Quarantine processing and establishment of cultures are necessary for host specificity testing and efficacy evaluation.

Giant Reed (*Arundo donax*) - The biological control program for *Arundo donax*, giant reed, received 28 shipments of the root-feeding armored scale, *Rhizaspidiotus donacis* (Hemiptera: Diaspididae) from Spain and France. Two shipments of the leafminer, *Lasioptera donacis* (Diptera: Cecidomyiidae) were also received. These agents were used for host specificity testing, efficacy evaluation, genetic characterization, and in the case of *R. donacis* for mass rearing and field release.

Mexican fruit fly (*Anastrepha ludens*) - The Mexican fruit fly (MFF) program has been maintaining and evaluating a wild culture of the MFF, *Anastrepha ludens*, which is known as the "Texas strain—Willacy County", for eventual introduction into the MFF mass rearing facility for strain replacement. Mating compatibility and competitiveness studies indicate that this strain is equal to or better than current strain.

Facility Maintenance - Plans were developed for restoring all of the quarantine greenhouses beginning in FY2012. The greenhouses will be individually and sequentially isolated, sealed-off, sanitized, and have the polycarbonate panels restored and resealed to ensure security and enhance plant and insect production. PPQ Officers will periodically Inspect and monitor progress of renovations.

Table 1. List of organisms with active permits for movement into PPQ Arthropod Quarantine - Edinburg, TX.

Permittee Name	Permit #	Date issued	Date Expires	Insect Name	Shipment Origins
Jorge M. Gonzales	P526P-09-02123	07/01/2009	7/01/2012	*Anastrepha ludens*	Texas
Kristy Gottfried	P526P-10-03418	10/18/2010	10/18/2012	*Anastrepha ludens*	Guatemala
Matt Ciomperlik	P526P-09-00940	04/21/2009	4/21/2012	*Aulacida acroptilonica*	Turkey, Uzbekistan
Leeda A. Wood	P526P-09-02428	07/29/2009	7/29/2012	*Tamarixia radiata*	Pakistan
Serguei Triapitsyn	P526P-09-03526	11/23/2009	11/23/2012	*Diaphorina citri*	Texas
Leeda A. Wood	P526P-09-03768	12/16/2009	12/16/2012	*Unidentified Hymenoptera*	India, China, Asia
Dr. John A. Goolsby	P526P-09-00905	04/15/2009	04/15/2012	*Tetramesa romana*	Spain, France
Leeda A.Wood	P526P-10-00058	01/06/2010	1/06/2013	*Unidentified entomopathogenic bacteria*	China, India, Asia
Dr. John A. Goolsby	P526P-10-03111	09/15/2010	09/15/2013	*Cryptonevra spp.*	France, Spain
				Lasioptera donacis	France, Spain
				Rhizaspidiotus donacis	France, Spain
				Tetramesa romana	France, Spain
Dr. John A. Goolsby	P526P-10-03999	12/16/2010	12/16/2013	*Rhizaspidiotus donacis*	Texas
Dr. Daniel Flores	P526P-11-03128	08/22/2011	08/22/2014	*Tamarixia radiata*	Texas

Table 2. Summarization of archived information for FY2011 on authorized personnel incoming and outgoing, materials and supplies incoming and outgoing, autoclaved and sterilized materials outgoing, foreign consignments incoming, beneficial insects permitted for outgoing, and log of final destination of released organisms.

Fiscal Year	FY11 Total
Number of Entries for Authorized Personnel	6550
Number of Incoming Materials into Quarantine	364
Number of Outgoing Materials from Quarantine	308
Number of Outgoing Autoclaved and Sterilized Materials for Disposal	220
Number of Incoming Foreign Consignments - Form AD-941 (Yellow)	8
Number of Incoming Foreign Consignments - Alternate Form AD-941 (Blank)	0
Number of Outgoing Permitted Beneficial Insects - Form AD-942 (White)	31
Number of Records for Final Destinations of Released Organisms (NonQuarantined) - Form AD-943 (Green)	1

Assessment, Identification, and Suppression of Microbial Contaminants in the Mexican Fruit Fly Mass Rearing Facility

CPHST STAFF: Bacilio Salas (lead), Hugh E. Conway (support), and Don Vacek (support)
TECHNICAL SUPPORT: Armando Loya
CHAMPION: Stuart Kuehn (SPHD-TX)
CONTACT: Bacilio Salas (bacilio.salas@aphis.usda.gov)
PROJECT: M7MO4

Accomplishments

- Documented baseline data for bacteria and yeasts present in Mexican fruit fly from Guatemala.
- Determined for the first time that *Morganella* sp. isolated from dead larva of Mexican fruit fly from Guatemala is pathogenic and detrimental to *A. ludens*.

Bacteria and yeasts from Guatemala

Anastrepha ludens the Mexican fruit fly (MFF) can cause serious economic losses to citrus producers in the in the Lower Rio Grande Valley-TX due to quarantines, lost markets, treatment costs, and yield reduction. Microbial contamination of eggs, larvae, and diet of *A. ludens* can halt the artificial rearing of MFF. A continuous monitoring of microbes during rearing is necessary to mitigate and curtail any detrimental effect.

To avoid the introduction of deleterious microbes via MFF eggs produced at the APHIS fruit fly facility in Guatemala, isolations were made from eggs at arrival and from larvae after rearing on artificial diet. Difco Pseudomonas Agar F (amended with Bravo WS), Serratia medium, Xylose Lysine Desoxycholate (XLD), and Sabouraud agar were used to detect *Pseudomonas aeruginosa*, *Serratia marcescens*, enterobacters, and fungi, respectively. Bacteria and yeasts were identified by using polymerase chain reaction (Accugenix, 223 Lake Dr. Newark, DE 19702).

Twenty microbial species were identified from eggs or larvae from Guatemala (Table.1). Fifty distinct morphotypes are still pending for identification. Most species appeared to be associated with eggs than with larvae. The troublesome species for insect rearing, *P. aeruginosa* and *S. marcescens,* were found at both facilities. Ten microbial species found in MFF from Guatemala were not found in MFF from Edinburg-TX (Table. 1).

Pathogenicity of *Morganella* sp. isolated from dead MFF larva

Unusual, dead, black, larvae were found while separating larvae from diet. To determine the cause of mortality, samples of dead larvae were sent to Mississippi State, where all isolates were identified as *Bacillus* species. Bacteria isolated at CPHST Mission Laboratory were sent to ACCUGENIXS for identification. Results showed that *Bacillus cereus*, *Providencia* species, and a *Morgenella* sp. were associated with dead larvae. In small diet cups, pathogenicity tests measuring survival from a specific larval instar stage to pupal stage showed that *Morgenella* sp. can cause high mortality (85-90%) if inoculated at 1st instar stage, some mortality (35-40%) at 2nd instar, and a little mortality (5-10%) at 3rd instar, compared to control. This differential effect on larval instar stage was independent of concentration of *Morganella* sp. inoculum used in the tests (Fig. 1). This is the first finding of *Morganella* sp. as pathogen of MFF, and further identification to species levels is being pursued.

Table 1. Bacteria yeasts isolated from Mexican fruit Fly (*A. ludens*) reared in APHIS fruit fly facility in Guatemala, contrasted with microbes found in Edinburg-TX facility.

Species of bacterial or yeast	Edinburg-TX					Guatemala	
	Eggs[1]	Larva[2]	Diet[3]	Air[4]		Eggs	Larva
Bacillus cereus	+[5]	-	-	-		-	+
Candida krusei	-	+	-	-		-	+
Chromobacterium haemolyticum	-	-	-	-		+	-
Cryptococcus humicola	-	-	-	-		+	-
Enterobacter asburias	-	-	-	-		+	-
Frigoribacterium faeni	-	-	-	-		+	-
Micrococcus luteus	-	-	+	-		-	+
Morganella sp.	-	-	-	-		-	+
Myroides odoratimimus	-	-	-	-		+	-
Pediococcus pentosaceus	-	-	-	-		-	+
Providencia sp.	-	+	-	-		-	+
Pseudomonas alcaligenes	+	-	-	-		+	-
Pseudomanas aeruginosa	+	-	-	+		+	-
Pseudomonas monteilii	+	-	-	-		+	-
Pseudomonas moselii	-	-	-	-		+	-
Pseudomonas otitidis	-	-	-	-		+	-
Pseudomonas sp.	+	+	+	+		+	-
Pseudomonas taiwanensis	+	-	-	-		+	-
Serratia marcescens	+	+	-	+		+	-
Stenotrophomonas maltophilia maltophilia	-	-	-	-		+	-

[1]Microbes on the surface of MFF eggs before placement on the MFF diet
[2]Microbes on the surface of larva after separation from the artificial MFF diet
[3]Microbes in the artificial MFF diet after separation from larvae
[4]Microbes in indoor-air near the area of extraction of MFF larvae from diet
[5]Signs: + = presence, - = absence

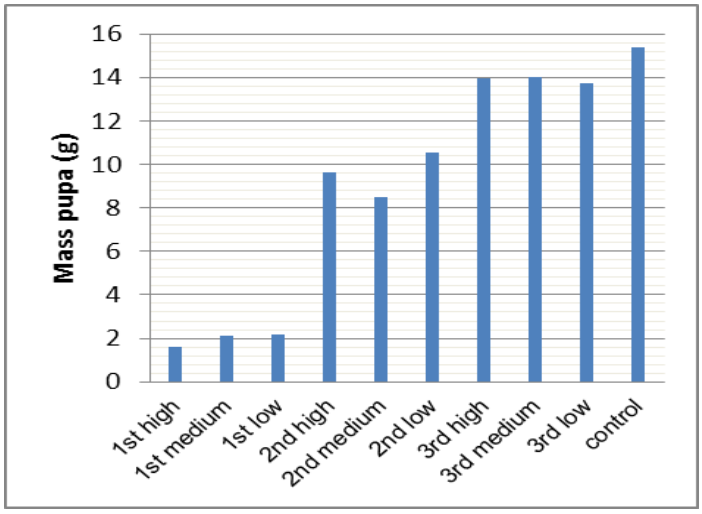


Fig. 1. Effect of *Morgenella* sp. on yield of MFF pupa after inoculation of 1st, 2nd, and 3rd instar of MFF larvae with three concentrations of the pathogen. (High, medium, and low concentrations are 6.0, 3.0, and 1.5 x 10^6 colony forming units per ml, respectively; two replicates per instar-concentration combination and three replicates for control.)

Fig. 2. Note the yellow colonies of *Morganella* sp. on XLD medium (left plates).

Mexican Fruit Fly Projects:

Mexican Fruit Fly Diet Development & Rearing Support, Operations Support for Fruit Fly Preventative Release and Eradication Program, Evaluation of New Fruit Fly Trap/Lure/Trapping Agent Combinations

CPHST Staff: Hugh Conway
Technical Support: Mateo Hernandez, Juan Veliz, Gilberto Salinas (4 m), and Oscar Reyes (2 m)
Co-operative agreement: UT Pan Am Dr. Christopher Vitek with Lab. Tech. Kristen Hopperstad
Champions: Mike Stefan, John Stewart, Wayne Burnett, (USDA)
Contact: Hugh Conway (hugh.e.conway@aphis.usda.gov)

Accomplishments:

- Completed egg incubation testing comparing methodologies (strip, bubbled, shaker) and diet testing comparing buffering agents in diet using citric vs hydrochloric acid
- Tested Guatemalan bubbled eggs (72h and 96h) against Texas MFF eggs
- Conducted quality control testing of Guatemalan black pupa (male predominate) strain including mating compatibility and competitive testing against three Texas fly strains
- Sampled Brush-land as host reservoir and summer field sampling of missed citrus fruit looking for potential over-summering source locations for ferile *Anastrepha ludens*
- Evaluated Mexican fruit fly aircraft and ground release machines
- Conducted field and laboratory bioassay tests of pesticide treatments (malalthion, NuLure, and GF-120 Spinosad) efficacy in the Mexican fruit fly program
- Evaluated effectiveness of two-component lures and torula yeast used in program trapping
- Conducted field cage studies on bait stations for potential use in dooryards
- Constructed a Mexican fruit fly monitoring tool allowing fruit fly coordinators and decision makers to analyze effectiveness of sterile releases in the MFF program areas

Guatemalan bubbled eggs testing

Guatemalan bubbled eggs were obtained from Carlos Caceres, Mexican fruit fly Mass Rearing Director, from Guatemala and tested in the CPHST Methods Development Building. Comparison testing indicated that the 72h bubbled in Guatemala produced more pupa per 6.5 kg tray of diet than other tested methods. Currently testing is being conducted in the Mass Rearing Facility with Guatemalan MFF eggs.

Guatemalan black pupae evaluation

Mexican fruit fly new strain development testing is being conducted at CPHST Methods Development Building for the Guatemalan black (male predominate) strain. Males have darker pupae color and can be preferentially selected by an optical sorter. Black pupae from Guatemala met or exceeded all IAEA quality control standards for pupa mass, percent emergence, percent flight ability, and survivability in 72 hour stress test. The ratio of male to female is high enough to make black pupae a promising program measure. In mating compatibility and competitive testing, the black pupa strain flies were compatible with all other

Table 1 Results of Guatemalan bubbled egg comparison tests

Treatment method	Yield pupa / tray (g)	Pupa size (mg)	Production / tray
Guatemala 72h+20h TX	660.8 ± 11.2	18.73 ± 0.26	35,280 pupae
Methods back-up	665.0 ± 9.3	19.29 ± 0.27	34,474 pupae
Guatemala 96h+1h TX	588.3 ± 13.2	19.23 ± 0.29	30,481 pupae
Guatemala 96h+0h TX	493.6 ± 17.9	19.97 ± 0.28	24,717 pupae

strain and more competitive than current strain and backup strain but not as competitive as Willacy strain. Additional testing for survivability length in field cages and dispersal is planned in 2012.

Monitoring tool for Mexican fruit fly release program

A monitoring tool was constructed for calculating flies per trap per day (FTD) and MFF over-flooding values to be used in the Mexican fruit fly Preventive Release Program (PRP). The tool allows fruit fly coordinators and decision makers to analyze for the first time the effectiveness of sterile releases in the program areas. The tool will be used for ongoing quality assurance of the sterile release program and to aid in decision making of the initiation of quarantines when fertile females are found to be mated.

Fruit fly frap/lure/trapping agent combinations testing in south Texas

Trapping tests were conducted to determine which trap lure combination captures the largest number of Mexican fruit flies. Tests include 2-component lures (cones, patches, pouches, and sponges) compared to torula yeast borax. Tests are evaluated over time (6 week periods) with replications. Fruit fly trap/lure/ trapping agent testing insures the Mexican fruit fly trapping program uses the best available tools for monitoring fly populations and evaluating the effectiveness of sterile releases.

Fig. 1 Kristen Hopperstad with adults from black pupa having ratio of 1 female to 614 males

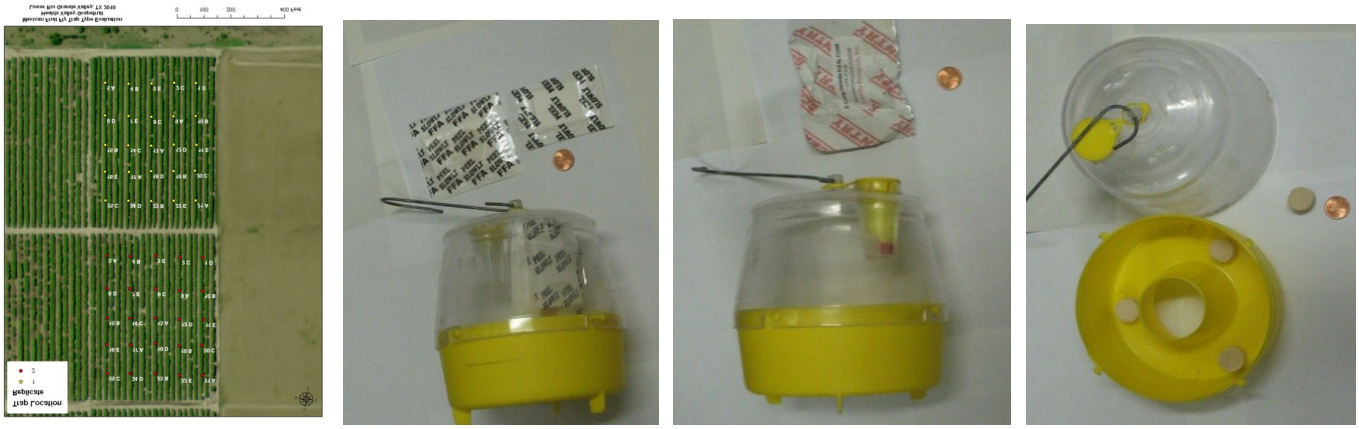


Fig. 2 Latin square design (5X5) with two replicates and three lures “1) 2-patches, 2) cone, and 3) torula yeast borax pellets” tested in 2-piece plastic biolure® trap

Biological Control of Harrisia Cactus Mealybug (*Hypogeococcus pungens*)

CPHST STAFF: Matthew Ciomperlik
CHAMPION: Leyinska Wiscovitch, PR SPHD
CONTACT: Matthew Ciomperlik
(matt.a.ciomperlik@aphis.usda.gov)

Accomplishments:

- Determined ovipositional behaviors of Leptomastidea on Hypogeococcus pungens.
- Conducted choice and non-choice host range tests of Leptomastidea on nine pseudococcid species.
- Leptomastidea sp. from Puerto Rico was shown to be host specific to H. pungens.

The Harrisia cactus mealybug, *Hypogeococcus pungens* (Hemiptera: Pseudococcidae) is a recent invasive and destructive pest of columnar cacti in Puerto Rico. Surveys since 2005 have found that *H. pungens* (HCM) is rapidly spreading in Puerto Rico's dry forests, and is now distributed in an area of about 1,500 km^2 on the main island (Fig 1). It has been responsible for causing significant galling damage and death of affected cacti in 13 municipalities, and will most likely spread to an additional 8 municipalities.

Figure 1. Distribution of *HCM* in Puerto Rico.

Survey activities during 2010 indicate that *H. pungens* is now common in southern Puerto Rico, and is distributed in most dry districts. Heaviest infestations are found in Cabo Rojo Guánica, Lajas, and Salinas. Areas with 100% infestation are common. HCM has not yet been found in Mona, Desecheo, Vieques, or Culebra Islands. Surveys of the affected areas indicate moderate to severe infestations of several key columnar cacti (e.g., *Leptocereus quadricostatus, Pilosocereus royenii, Melocactus intortus, Stenocereus fimbratus,* and *Cereus hexagonus*). The affected areas are remote and/or part of the Guanica Dry Forest Preserve; therefore, chemical control options are not practical. In this case, classical biological control appears to offer the best management alternative for this pest, hopefully precluding its further spread into the continental U.S.

Figure 2. Galls on *P. royenii* caused by *HCM*.

In 2011, a graduate student Emmanuel Vélez, evaluated the biology of *Leptomastidea* nr. *antillicola* Dozier (Hymenoptera: Encyrtidae), a parasitoid natural enemy of HCM, and reported on methods for rearing both the host and parasitoid in Velez and Segarra (in press). Development times for parasitoid (egg-adult) were 19 to 30 days, with 90% of adults emerging by day 25. Sex ratios in laboratory rearedand field collected parasitoids were similar and slightly female-biased, 1.2:1 (females: males). Median male emergence occurred at 21 days, a day earlier than median emergence in females. Velez and Segarra likewise reported on mating behavior of *L.* nr. *antillicola*.

Emmanuel Vélez, as part of his master's thesis under the supervision of Dr. Alex Segarra, observed ovipositional behaviors of the native PR *Leptomastide* (Figure 3) in non-choice tests and conducted host range tests on nine different pseudococcid species. The pseudococcid's chosen for the experiments included:

Maconellicoccus hirsutus, *Paracoccus marginatus*, *Planococcus citri*, *Dysmicoccus brevipes*, *Ferrisia virgata* which were reared on potato tubers. *Nipaecoccus nipae* was reared on coconut palms, *Pseudococcus longispinus* and *Puto barberi* were reared on *Acalypha* sp. plants. In the laboratory non-choice tests, ten experienced 24 h old female *Leptomastidea* were caged with individual plants containing a single pseudococcid host. Observations of oviposition behaviors showed that *Leptomastidea* walked over and antennated individuals of *Dysmicoccus brevipes, Maconellicoccus hirsutus, Nipaecoccus nipae, Paracoccus marginatus, Planococcus citri,* and *Puto barberi,* but attempted no oviposition on those pseudococcids. *Leptomastidea* did attempt to oviposit in long-tailed mealybug (*Pseudococcus longispinus*), but appeared to have problems with the dorsal and lateral spines and did not successfully parasitize the host. Surprisingly, *Leptomastidea* failed to parasitize *Ferrisia virgata*, which was the host reported by Dozier (1937) in his original description of *Leptomastidea antillicola* in Puerto Rico.

In host range tests, *Hypogeococcus pungens* on *Alternanthera* plants were provided in all tests while a second host (choice test) varied to those listed above. The choice tests were performed by isolating 10 female *Leptomastidea nr antillicola* for 72 hours in the presence of both hosts, and replicated three times per combination of hosts. Each host plant contained a minimum of 100 mixed stages of the host pseudococcid being tested. In the choice tests, *H. pungens* was readily parasitized and adult *Leptomastidea* emerged from the host. None of the alternate species of hosts were parasitized by *Leptomastidea*. These tests suggest that the native *Leptomastidea nr. antillicola* shows a high degree of specificity in attacking *Hypogeococcus pungens*, even in the presence of other taxonomically close pseudococcids as is *Nipaecoccus nipae*.

Genetic analysis comparing *Leptomastidea* populations from Puerto Rico, Barbados, and Florida are still pending at this time. A delay in hiring a post-doc to conduct the DNA sequencing will likely delay comparisons of the populations until well into 2012.

Collaborators:

Aixa Ramirez-Lluch, CAPS Coordinator, Oficina Estatal de Muestros (PR Dept. of Agriculture), Santurce, PR

Dr. Alejandro Segarra, University of Puerto Rico, Agricultural Experiment Station, Mayaguez, PR

Emmanuel Vélez -Perez, University of Puerto Rico, Agricultural Experiment Station, Mayaguez, PR

Norberto Gabriel, Rosimar Morales-Mallery, and Hector Rivera; USDA APHIS PPQ Puerto Rico Work Unit, San Juan, PR

Dr. Jose Carlos Rodriguez, University of Puerto Rico, Agricultural Experiment Station, Rio Piedras, PR.

Figure 3. Adult *Leptomatidea*, parasitoid of HCM, female (left) and male (right) (photo: Antonio Francis, PRDA)

Rehydration of Frozen Boll Weevils for Quality Control Measures in the Texas Boll Weevil Eradication Program

CPHST STAFF: Jason Carlson (Lead), Leeda Wood (Lead), David Bartels (Support)
CHAMPION: Larry Smith, Texas Boll Weevil Eradication Program Inc.
CONTACT: Jason Carlson (jason.carlson@usda.aphis.gov)

Accomplishments

- Developed a method to rehydrate and mark dried boll weevils to help aid with quality control measures in the Texas Boll Weevil Eradication Program.

Providing a Supply of "fresh" boll weevils

The cotton boll weevil (*Anthonomus grandis* Boheman) is the most destructive pest of cotton in Texas and the U. S. (Figure 1). Boll weevil eradication and management programs started in 1958 after the National Cotton Council realized the economic toll boll weevils had on domestic cotton production. A USDA Boll Weevil Research Lab was created followed by boll weevil eradication experiments. Boll Weevil eradication measures continue to this day, and have eradicated boll weevil populations in most of the United States, with only a few cotton producing areas remaining. Monitoring for boll weevils is a very important first step for boll weevil eradication and is used as a guide to implement boll weevil control strategies.

Figure 1. The cotton boll weevil.

Monitoring for boll weevil is done by placing pheromone traps adjacent to cotton fields and along fence lines and roads in cotton growing areas. For quality control of the monitoring process, previously collected and frozen boll weevils are marked with a fingerprint powder that is not visible to the human eye, but fluoresces when place under black light. Program supervisors will randomly spike boll weevil traps with marked weevils to monitor the program trappers. When the boll weevils samples are collected and brought back to the lab for identification, this fluorescing powder differentiates the quality control weevils from natural weevils caught in traps. One problem, however, is that dead boll weevils placed in traps can desiccate rapidly, rendering them fragile and prone to breaking, which makes identification difficult. Our objective was to determine if boll weevils could become rehydrated and how long boll weevils could remain pliable and intact after rehydration and still fluoresce. Experiments were performed at two different temperatures; 27˚C (Laboratory conditions) and 32˚C (greenhouse conditions). A hand sanitizer for rehydration and three different fluorescing markers were tested to determine the duration of boll weevil pliability and fluorescence. At 120 hours, 46% of the boll weevils treated FR-HS-FP kept at 27˚C were pliable. At 168 hours, 13% of the boll weevils treated FR-FP-HS and DR-FP-HS kept at 32˚C were pliable (Tables 1 and 2). Statistical comparisons were not done on any of the fluorescent markers because all boll weevils retained the three applied fluorescent markers throughout each experiment.

Treatments	N		0h	24h	48h	72h	96h	120h	144h
		Fro-zen							
FR-FP-HS	15		100	100	93	53	6	6	0
FR-CO-HS	15		100	100	100	53	46	26	0
FR-MCO-HS	15		100	100	93	80	66	20	0
FR-HS-FP	15		100	100	100	86	80	46	0
FR-HS-CO	15		100	100	100	86	46	6	0
FR-HS-MCO	15		100	100	100	53	33	13	0
		Dried							
DR-FP-HS	15		100	100	86	66	53	26	0
DR-CO-HS	15		100	100	93	46	13	6	0
DR-MCO-HS	15		100	100	100	73	40	6	0
DR-HS-FP	15		100	100	93	66	46	13	0
DR-HS-CO	15		100	100	100	73	46	33	0
DR-HS-MCO	15		100	60	60	53	40	13	0
Control	15		100	46	46	13	0	0	0

Table 1. Percentage of boll weevils remaining pliable at different time intervals under laboratory conditions at 27˚C in experiments conducted at Mission Laboratory, Edinburg, TX. November 2011.

Treatments	N		0h	24h	48h	72h	96h	120h	144h	168h
		Frozen								
FR-FP-HS	15		100	100	93	86	66	60	6	13
FR-CO-HS	15		100	100	66	13	6	6	6	0
FR-MCO-HS	15		100	100	60	46	26	26	13	0
FR-HS-FP	15		100	100	73	73	40	40	40	6
FR-HS-CO	15		100	100	46	26	20	6	6	0
FR-HS-MCO	15		100	100	60	60	46	40	40	6
		Dried								
DR-FP-HS	15		100	100	93	93	60	40	40	13
DR-CO-HS	15		100	100	66	40	20	6	6	0
DR-MCO-HS	15		100	93	40	26	6	6	6	0
DR-HS-FP	15		100	100	80	80	40	40	26	6
DR-HS-CO	15		100	100	60	26	20	20	13	0
DR-HS-MCO	15		100	80	53	26	13	13	6	0
Control	15		100	46	20	0	0	0	0	0

Table 2. Percentage of boll weevils remaining pliable at different time intervals under greenhouse conditions at 32˚C in experiments conducted at Mission Laboratory, Edinburg, TX. November 2011.

CPHST Mission Laboratory Personnel

Standing Left to Right: MD Sajedul Islam, Dan Flores, Dina Suarez, Raul Ruiz, Roxanne Farris, Matt Ciomperlik, Oscar Obregon, Aaron Flores, Hugh Conway, Joe Martinez, Don Vacek, Rosie De Leon, Elena Ibanez, Kristen Hopperstad, Evan Braswell, Josie Salinas, Dante Narvaez, Joe Renteria, Andrew Parker, Johnathan Waggoner, Danny Martinez, Roxanna Ramos, Emily Sanchez, and Armando Loya.

Sitting Left to Right: Bacilio Salas, Mateo Hernandez, David Bartels, and Johnny Rodriguez.

Norman Barr
Molecular Biologist

Hugh Conway
Entomologist

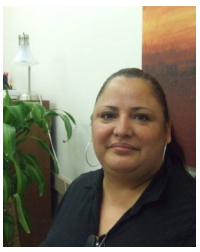
Elena Ibanez
Laboratory Support Assistant

David Bartels
Entomologist

Roxanne Farris
Geneticist

Fritzie Into
UTPA Technician

Evan Braswell
Molecular Biologist

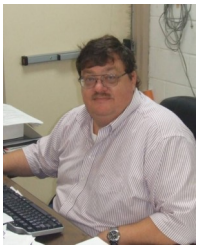


Rosie De Leon
Biological Science Technician

MD Sajedul Islam
UTPA Post-Doctoral Scholar

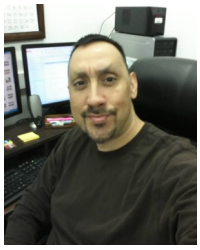
Jason Carlson
Biologist

Aaron Flores
Insect Production Worker

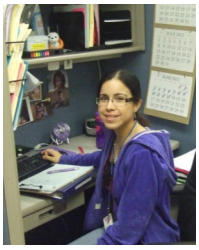


Steven Kirpes
IT Specialist

Janet Casas
Office Automation Clerk



Dan Flores
Entomologist



Lisa Ledezma
Biological Science Technician

Albino Chavarria
Supervisory Biological Science Technician

Mateo Hernandez
Biological Science Technician

Armando Loya
Biological Science Technician

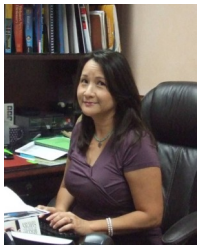
Matt Ciomperlik
Laboratory Director

Kristen Hopperstad
UTPA Technician

Danny Martinez
Biological Science Technician

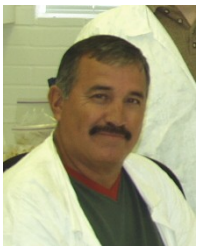
Joe Martinez
Biological Science Technician

Eustorjio Rivas Jr.
Biological Science Technician



Dina Suarez
Administrative Support Assistant

Dante Narvaez
High School Summer Intern

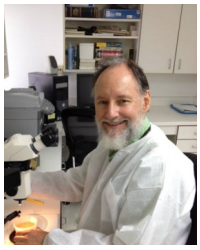


Johnny Rodriguez
Biological Science Technician

Terrance Todd
Biological Science Technician

Oscar Obregon
Biological Science Technician

Raul Ruiz
Geneticist



Don Vacek
Biologist

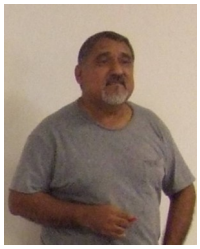
Andrew Parker
Biological Science Technician

Bacilio Salas
Plant Pathologist

Danny Vasquez
Biological Science Technician

Oscar Pizano
Biological Science Technician

Gilbert Salinas
Biological Science Technician



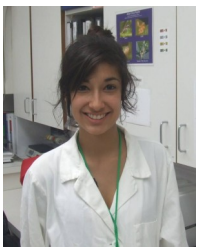
Juan Veliz
Biological Aid

Roxanna Ramos
UTPA Student Intern

Josie Salinas
Biologist

Johnathan Waggoner
UTPA Student Intern

Jose Renteria
Biological Science Technician



Emily Sanchez
UTPA Student Intern

USDA APHIS PPQ CPHST

Mission Laboratory

22675 N Moorefield Rd # 6414

Edinburg, TX 78541-5033

PHONE: (956) 205-7668

FAX: (956) 205-7680