Florida Citrus Advanced Technology Program

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*	Psyllid	1/12/10

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

HLB Pathogen Confirmed in Psyllid Digestive System (Filter Chamber and Alimentary Canal) and Salivary Glands

TITLE and CONTACT INFORMATION

Proposal Title Pathogen-Vector Relations between Asian Citrus Psyllid and Liberibacter asiaticus

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Organization USDA-ARS	*Total Direct Funds (current year)* $148,207

REPORT UPDATE (650 words; provide details about your headline)

1: Localization of Liberibacter asiaticus (Las) in internal organs of Asian citrus psyllid (ACP). In order to study the cellular interactions of Las in its psyllid vector, the following four methodologies have been used for localization of this bacterium in dissected organs and hemolymph smears of ACP and in leaf sections and extracts of HLB-diseased plants: A. Immunofluorescence confocal laser scanning microscopy. Two polyclonal and several monoclonal antibodies prepared against Las membrane proteins have been tested at various dilutions (1/20 to 1/400) and incubation times (3-27 hrs). Some of the monoclonal antibodies produced positive fluorescent labeling in the phloem area in sections of HLB- infected citrus leaves and in hemolymph smears of psyllids collected from HLB-infected trees, but not in whole-mount insect organs. These results are encouraging but appear to indicate that such antibodies may be slow to penetrate whole insect organs. Efforts are now underway to resolve this issue with various permeabilization procedures or by immunolabeling of paraffin sections rather than whole organs. B. Fluorescence In situ hybridization (FISH). The oligonucleotide primer tested so far is based on the following sequences (20 bases) of Las, tagged with Alexa Fluor 488: TCGAGCGCGTATGCAATACG. Several FISH protocols have been tested on dissected organs of ACP and on leaf sections and extracts from HLB-diseased plants. Green fluorescence, indicating Las, was detected in the filter chamber and midgut of field-collected ACP, but not in healthy controls. It was also detected in leaf sections and plant extracts from HLB-diseased plants but not in those from healthy plants. We are continuing to refine the FISH procedure and to test two other recently-designed Las primers . C. Quantitative RT-PCR of dissected insect organs. We tested two different RT-PCR procedures for detection of Las in dissected salivary glands, alimentary canals and other parts of individual ACP adults. In two successive experiments, using DNeasy Kit for extraction of DNA, Las was detected in 17-33% of the salivary glands, 27-47% of the alimentary canals, and in 47-53%of the rest of the body of psyllids collected from HLB-infected trees in Fort Pierce, FL. during November and December 2009. To our knowledge, this is the first direct demonstration of Las (using PCR) in the alimentary canal and salivary glands of ACP. D. Transmission electron microscopy (TEM) We are currently using a combination of TEM and RT-PCR, comparing the ultrastructure, including the bacterial fauna, of psyllid adults that have never been exposed to infected plants with those collected from HLB-infected trees and tested positive for Las with RT-PCR. Fixation and embedding of samples have been done, while thin-sectioning and TEM observations are underway, providing the base for TEM-immunolabeling studies.
2: Clarification of various acquisition and transmission parameters between ACP and Las. [A] Limited success has thus far been achieved using an artificial diet-in vitro feeding method for Las detection in live ACP. Further improvements in this technique are continuing. [B] Among adults field collected from Las-infected trees, averages of 37% females and 38% males have tested positive by PCR. No differences in infectivity among the three color morphs of adult ACP have been noted. [C] To assess the incubation period of Las in young citrus with respect to testing PCR-positive, groups of field-collected adult ACP were caged on individual citrus plants for 5 days and then assayed individually for Las. Plants that had been infested by Las-infected ACP were then PCR assayed weekly. Among trees infested by at least one infected ACP, 10%, 25%, and 50% tested positive within 4, 6 and 31 weeks, respectively. No relationship was found between the number of infected ACP per plant and the incubation period of Las. [D] There was no evidence of sexual transmission of Las between males and females of ACP. [E] In an acquisition study, greater percentages of females acquired Las than males.