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	5/3/10

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

Detection of Liberibacter asiaticus in the hemolymph, alimentary canal and salivary glands of the Asian citrus psyllid

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 the hemolymph and other tissues and organs of the Asian citrus psyllid (ACP). The following four methodologies have been tested and used with promising results. [A] Immunofluorescence confocal laser scanning microscopy: Two polyclonal and several monoclonal antibodies prepared against Las membrane proteins have been tested at various dilutions and incubation times. Some of the monoclonal antibodies produced positive labeling in the phloem area in sections of HLB- infected citrus leaves and in hemolymph smears of ACP collected from HLB-infected trees, but not in whole-mount ACP organs, suggesting that such potentially useful antibodies may be too slow to penetrate whole insect organs. We are working to resolve this issue with various permeabilization procedures or by immunofluorescent labeling of paraffin sections rather than whole organs. [B] Fluorescence In situ hybridization (FISH) using three oligonucleotide primers: Several FISH protocols have been tested on hemolymph smears and dissected organs of ACP and on leaf sections and extracts from HLB-infected plants. Green fluorescence, indicating Las, was detected in the hemolymph, filter chamber and midgut of field-collected ACP, but not in healthy controls. It was also detected in leaf sections from HLB-infected citrus and periwinkle plants but not in those from healthy plants. We are continuing to refine our FISH protocols to reduce the background fluorescence and to increase the permeability of insect tissues. [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 five successive experiments, Las was detected in 13-24% of the alimentary canals, 12-16% of the salivary glands, and in 16-25% of the rest of the body from psyllids collected from HLB-infected trees in Fort Pierce, FL. This is the first direct demonstration of Las (using PCR) in the alimentary canal and salivary glands of ACP. We are currently testing the relative concentration of Las in various ACP organs. [D] We are using a combination of transmission electron microscopy (TEM) and RT-PCR to compare the ultrastructure of ACP adults that have never been exposed to infected plants with those collected from HLB-infected trees and are PCR-positive for Las. This is providing a very useful library on the ultrastructure of the alimentary canal, salivary glands and other organs of ACP as well as its bacterial symbiotes, and will form the basis for future immunogold-labeling TEM studies on Las pathogen-vector interactions in ACP at the cellular and tissue levels. 2. Clarification of various acquisition and transmission parameters between ACP and Las. [A] Among ACP adults field collected from HLB-infected citrus trees, averages of 37% females and 38% males have tested positive by PCR. Significantly higher percentages of field-collected adult ACP tested positive for Las during November 2009 (38.9%) than during late March 2010 (22.1%), and CT values associated with adults collected during November (mean 24.8) were significantly lower than for adults collected during late March 2010 (mean 30.5). [B] An average of 16% individual HLB-infected adult ACP (field-collected, mean CT of 26.4) transmitted HLB to young potted citrus trees. Taken together, the data in1C and 2B above support our hypothesis that transmission barriers to Las in ACP (including the alimentary canal and salivary glands) may play an important role in HLB transmission, since much higher percentages of ACP adults were PCR-positive compared to those that were actually able to transmit HLB to citrus plants. Our studies are continuing to elucidate the roles of these and other psyllid organs/ tissues in HLB transmission by ACP.