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	10/13/10

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

LAS probably multiplies or accumulates in the 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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Phone 772-462-5897	*Project Duration (years)* 3 *Year of Project* 2
Organization USDA-ARS	*Total Direct Funds (current year)* $148,207

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

Objective 1. Localization of Liberibacter asiaticus (LAS) in the hemolymph and other tissues and organs of the Asian citrus psyllid (ACP). [A] Using Fluorescence In situ hybridization (FISH): We continued our studies to improve the sensitivity and specificity of several FISH protocols to localize LAS in hemolymph smears and dissected organs of ACP, and in leaf sections from HLB-infected plants as positive controls. So far, LAS has been detected in the hemolymph, filter chamber and midgut of LAS-infected ACP both from laboratory colonies and infected field trees, but not in healthy controls. It has been also detected in leaf sections from HLB-infected citrus plants but not in those from healthy ones. [B] Using quantitative RT-PCR on dissected insect organs: We tested different RT-PCR protocols for detection and quantification of LAS in dissected salivary glands, alimentary canals and other parts of individual ACP adults either from our laboratory colony or collected from field HLB-infected citrus trees. In field collected psyllids, the proportion of infected salivary glands (47%) was significantly lower than those of the alimentary canal (72%) or other body parts (79%). A similar trend was found with ACP adults reared as nymphs in our lab. on HLB-infected citrus. Interestingly, the relative titer of LAS, compared to psyllid genomic DNA in each ACP sample, was significantly higher in both the salivary gland and alimentary canal compared to that in the rest of the insect body. These results strongly suggest that the salivary glands constitute a major transmission barrier to LAS in the psyllid vector, and that LAS may replicate or accumulate in both the alimentary canal and salivary gland of ACP. [C] We continue to use 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 citrus 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 studies on LAS pathogen-vector interactions in ACP at the cellular and tissue levels.

Objective 2. Clarification of various acquisition and transmission parameters between ACP and LAS. [A] We have started two large experiments to compare between young (2nd-3rd-instar) nymphs and adults having various acquisition access periods (AAP) on HLB-infected citrus plants. Previous reports have indicated that ACP nymphs are more efficient in LAS acquisition than adults, and that LAS probably multiplies in ACP nymphs but not adults, but the effects of various AAPs on nymphal and adult stages in this regard have not been carefully studied so far. Our experiments are thus designed to study the effects of various AAPs on both acquisition and transmission of LAS in ACP, as well as on LAS replication in both nymphs and adults. [B] Percentages of LAS-infected psyllids tended to be lower during late spring or early summer with no other obvious seasonal trends during 2008-2009 at a USDA grove in Fort Pierce, FL. A general decline in percentages of infected psyllids appears to have taken place during 2009-2010. No seasonal differences in percentages of infected psyllids or titers of LaAS in psyllids have been found with respect to psyllid sex or color morph. Among infected (CT<36) psyllids from the grove, an average of 18% (range 0 - 64%) psyllids transmitted LAS to healthy seedlings in monthly experiments, with no seasonal trends identified. Transmission rates are being investigated for each sex and for each of the three color morphs.