Florida Citrus Advanced Technology Program

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

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2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	Psyllid	4/15/10

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

Detection of Las 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). [A]. Fluorescence In situ hybridization (FISH) studies are continuing using three oligonucleotide primers (including a newly designed one) based on the following sequences of Las: Primer 1 (HLBr): TCG AGC GCG TAT GCA ATA CG, Primer 2 (LL-REV): TCC CTA TAA AGT ACC CAA CAT CTA GGT AAA; Primer 3 (Cr-RDR-1): TAT ATT GTT CAC ATG AGG GAG CAT TTA ACC. Several FISH protocols have been tested on hemolymph smears and dissected organs of ACP and on leaf sections and extracts from HLB-diseased 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-diseased 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 permeabilization of insect tissues.
[B]. 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 an average of 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. To our knowledge, this is the first direct demonstration of Las (using PCR) in the alimentary canal and salivary glands of ACP. Additionally, percentage of infection did not differ significantly between the three insect parts in males but was significantly lower in the salivary glands than in the alimentary canals of females suggesting that in the latter the salivary glands may act as transmission barriers.
[C]. Transmission electron microscopy (TEM): We are continuing to use a combination of TEM and RT-PCR, comparing the ultrastructure 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. 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, tissue and organ 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 (not significantly different). 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 in 2A and 2B, combined with those in1B 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 proved PCR-positive compared to those that were actually able to transmit the disease to citrus plants. Our studies are continuing to elucidate the roles of these and other psyllid organs in HLB transmission by its vector.