CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

ACP salivary gland, a barrier to Las infection &/or transmission

Proposal Title

Pathogen-Vector Relations between Asian Citrus Psyllid and Liberibacter asiaticus

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
7/15/11	Citrus Research and Development Foundation	Unclassified

REPORT UPDATE (500 words; summarize your accomplishments)

Objective 1. Localization of Liberibacter asiaticus (Las) in the Asian citrus psyllid (ACP). [A] Our qPCR study on dissected ACP organs and tissues provided the first PCR confirmation of Las in the alimentary canal and salivary glands of ACP and strongly suggested that the salivary glands constitute an important infection and/or transmission barrier to Las in the psyllid vector. This partly explains why the proportions of PCR-positive psyllids are normally much higher than those that can actually inoculate Las into citrus plants. Our results (Ammar et al., 2011, Ann. Entomol. Soc. Amer. 104: 526-533) also suggested that Las may replicate or accumulate in both the alimentary canal and salivary glands of ACP. [B] We were finally able to study the distribution of Las in various organs and tissues of ACP by fluorescent in situ hybridization (FISH). Only one of the six protocols tested succeeded in the localization of Las in hemolymph smears and dissected organs of ACP adults that fed on Las-infected plants and in sections from infected leaves. Using specific DNA probes with FISH and confocal laser scanning microscopy, Las was detected in the filter chamber, midgut, Malpighian tubules, hemolymph, salivary glands, ovaries and in muscle and fat tissues of Las-infected ACP, as well as in the phloem of Las-infected leaves. Our results showed the near-systemic infection of ACP, which suggests that Las may replicate in various tissues of ACP, and confirmed our PCR results suggesting that the salivary glands constitute a barrier to Las infection and/or transmission. This study was recently accepted for publication (Ammar et al., 2011, J. of Phytopathology, in press).
Objective 2. Elucidation of various acquisition and transmission parameters between ACP and Las. [A] We have developed a new 'detached-leaf assay' method that potentially speeds up Las-inoculativity assays on ACP from the current 6-12 months, when using whole plants, to only 2-3 weeks when using detached leaves. Las-infected ACP adults were tested for inoculativity by caging them singly or in small groups (5 or 10 per leaf) in 50-ml polypropylene tubes on detached healthy citrus leaves for one week (as described by Ammar & Hall, 2011, Florida Entomologist 94:340-342). These leaves were then assayed by qPCR, 1-2 weeks later, using two Las primers. So far, the results of three trials on Las-inoculativity tests on detached leaves using the more conventional Li primers are largely comparable to those recently reported for Las transmission when whole citrus seedlings were used for inoculation. Our results indicate that detached citrus leaves can be used to test the inoculativity of single or small groups of ACP and that this new method can speed up inoculativity tests on citrus psyllids considerably, which should enhance various studies on HLB epidemiology and vector interactions. An extended abstract on this new method is published in "Proceedings of the 2nd. Inter. Res. Confer. on HLB held in Orlando, FL, Jan. 10-14, 2011". [B] We conducted two large experiments to study the effects of various acquisition access periods on Las-infected citrus plants (between 1 day and 2 wks) on both acquisition and transmission of Las by ACP nymphs and adults, as well as on Las replication in both life stages. We are still analyzing hundreds of individual psyllids from these two experiments with qPCR using primers to Las and to one of the psyllid genes for comparison. [C] In a field study on ACP adults collected from HLB-diseased citrus trees in Fort Pierce, FL since Feb. 2009, there have been no strong trends observed over time with respect to percentages of Las-infected ACP or titers of the pathogen. However, during late summer 2010, there were eight consecutive weeks in which percentages of ACP testing PCR-positive for the Las bacterium averaged below 10%. Statistical analyses indicated that increased rainfall and excessively high air temperatures during these weeks were significantly correlated with the lower percentages of Las-infected ACP.

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SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.