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	10/16/10

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

Effect of vector developmental stage on acquisition efficiency of Candidatus Liberibacter asiaticus

TITLE and CONTACT INFORMATION

Proposal Title Factors influencing acquisition and inoculation of Candidatus Liberibacter asiaticus by Diaphorina citri

Principal Investigator Joao Roberto Spotti Lopes	*PI Last Name* Lopes
Email jlopes@esalq.usp.br	*FDACS Contract Number* NAS ID Lopes-126
Phone +55 (19) 3429-4199, ext. 212	*Project Duration (years)* 3 *Year of Project* 2
Organization University of Sao Paulo	*Total Direct Funds (current year)* $63,200.00

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

Transmission of Candidatus Liberibacter asiaticus by the Asian citrus psyllid (ACP), Diaphorina citri, probably involves complex psyllid-pathogen interactions, which should be understood in order to improve management strategies to control HLB. The goal of this research is to determine factors that influence the risks of acquisition or inoculation of the pathogen (Candidatus Liberibacter asiaticus - CLas) by D. citri, e.g vector developmental stage, feeding periods, leaf age and symptom expression/bacterial population in disease plants, in order to optimize strategies to avoid or reduce disease spread within and between citrus groves. We already set up the first four of five proposed studies and the project is progressing as planned originally. Partial results of previous studies showed that bacterial acquisition can occur when ACP adults feed on asymptomatic infected plants, although acquisition efficiency is higher on citrus plants with higher bacterial titers, usually symptomatic. We also have reported that acquisition efficiency by psyllid adults is markedly affected by citrus leaf age and duration of acquisition access periods (AAP). Efficient acquisition depends on the availability of young leaves in infected plants and long AAPs (>2 days).

In this quarterly report, we present details of the first study, concluded in September/2010, which was carried out to determine how acquisition efficiency of CLas varies in relation to ACP developmental stages. Health insects of known age were obtained from a lab colony maintained on Murraya paniculata. In each experiment trial, groups of 40 adults with 3-5 days post-emergence and newly-molted nymphs of all development stadia (1st, 2nd, 3rd, 4th and 5th instars) were confined on young leaves of a same symptomatic branch of an infected plant, during a 48-h AAP. Psyllids of each treatment (developmental stage) were confined on a separate leaf. After the AAP, the insects were maintained on healthy sweet orange seedlings for 21 days (healthy seedlings were replaced after the first 14 days). Surviving psyllids were then fixed in 100% ethanol and individually tested for the presence of CLas by real-time quantitative PCR (qPCR). As a negative control, a group of 40 psyllids of the same rearing batch was transferred directly to the healthy seedlings (without previous AAP on infected plants). The experiment was repeated three times using distinct pathogen source plants. Acquisition efficiencies by 1st, 2nd, 3rd, 4th and 5th instars, as measured by the proportion of infective psyllids by qPCR, were 68.4, 60, 66.6, 84 and 65.3%, respectively. In contrast, only 42.9% individuals were infective when acquisition took place during the adult stage. Interestingly, a higher concentration of CLas was observed in psyllids that acquired the bacterium during the 4th or 5th instar. These results show that all developmental stages of ACP are able to acquire the bacterium with moderate to high efficiencies during a 48-h AAP on infected plants, but nymphs are more efficient than adults. We are now concluding PCR assays for detection of CLas in the test seedlings exposed to the psyllids of each treatment, to check if the higher acquisition rates by nymphs result in higher transmission rates of this pathogen.