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	7/15/09

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

Vector developmental stage and infected citrus leaf age influence acquisition efficiency of Liberibacter asiaticus

TITLE and CONTACT INFORMATION

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

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Organization University of Sao Paulo	*Total Direct Funds (current year)* $63,200.00

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

In this project, we are investigating how acquisition efficiency of Candidatus Liberibacter asiaticus by Diaphorina citri varies depending on vector developmental stage (study 1), plant phenology (young leaves x mature leaves) (study 2) and pathogen titer and symptom expression in source plants (study 3). We will also investigate the time period required for psyllid adults to inoculate the pathogen in healthy citrus (study 4) and if a systemic insecticide can affect this process (study 5). So far we have started experiments related to studies 1-4, and have partial results for studies 1 and 2, which are described below. The project is progressing as planned in the original proposal.

In study 1, we are comparing different D. citri nymphal stadia (1st, 2nd, 3rd, 4th and 5th instars) and adults (1 wk old) with respect to acquisition efficiency of Ca. L. Liberibacter on citrus. The experiment is being carried out in a climate-controlled room [25 ± 1oC, 60 ± 20% RH, and 14:10 h (light: dark) photoperiod]. A first trial was set up in May/09; 40 psyllid adults and nymphs of each instar were confined on distinct leaves of a young shoot of a symptomatic infected plant, with recently expanded leaves, inside leaf cages. After an acquisition access period (AAP) of 48 h, the insects of each age treatment were first transferred to healthy citrus seedlings for a latent period of 15 days, and then transferred to healthy test seedlings (5 insects/plant) for a 7-day inoculation access period (IAP). After the IAP, total DNA of each insect extracted and the sample was submitted to real-time quantitative PCR (qPCR). In this first trial, we got an infectivity rate of 50, 75 and 0% when acquisition occurred during the 1st instar, 5th instar and adult stage, respectively, indicating that nymphs acquire the bacterium more efficiently than the adults. A high mortality of 2nd, 3rd and 4th instars during the latent period precluded the analysis of their infectivity. Other two trials of this experiment will be set up in Aug/09 and Sept/09, when we hope to get data for all instars.

In the second study, we evaluated the preference of D. citri for different parts of a citrus plant, and the effect of leaf age (young and asymptomatic x symptomatic mature leaves) in source plants infected with Ca. L. asiaticus on acquisition efficiency and probing behavior of D. citri. This insect showed a clear preference for young leaves of the upper portion of the plant (71.3% of the individuals), and main vein on the abaxial leaf surface (87.1%). Groups of healthy lab-reared D. citri adults were then confined separately on a fully-expanded young leaf and on a mature (symptomatic) leaf of source plants of Ca. L. asiaticus. After an 4-day AAP, psyllids from each treatment were kept on healthy plants for 24-day latent period and then tested for infectivity by qPCR. D. citri acquired Ca. L. asiaticus with greater efficiency on young (asymptomatic) leaves (49.5%; n=4) than in mature (symptomatic) leaves (0%; n=4) from infected plants. We plan to repeat this experiment and include a third treatment (not fully-expanded young leaf). To investigate possible reasons for the differences in acquisition efficiency, we analyzed the probing behavior of adult females of D. citri on mature x young leaves of infected citrus plants by the Electrical Penetration Graph (EPG) technique. Phloem ingestion was longer and observed more often on young leaves. Within 5 h, around 50% individuals on young leaves started sustained phloem ingestion (E2), whereas less than 15% individuals on mature leaves did so. On mature leaves, the insects spent most of the time with the stylets in the parenchyma (pathway phase) or non-probing. The higher frequency and longer duration of phloem ingestion appears to explain at least in part the higher acquisition efficiency of Ca. L. asiaticus when D. citri is confined on young asymptomatic leaves.