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)

Vector feeding time affects acquisition of greening pathogen

Proposal Title

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

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
1/15/11	Citrus Research and Development Foundation	Vector Management

REPORT UPDATE (500 words; summarize your accomplishments)

The literature shows that transmission efficiency of Candidatus Liberibacter asiaticus (CLas) by the Asian citrus psyllid (ACP), Diaphorina citri, can be quite variable (1-100%). We are conducting a series of studies to determine the impact of various factors on the efficiency of acquisition of CLas by ACP), such as vector development stadia and duration of acquisition access period (AAP) (study 1), as well as leaf age (study 2), pathogen titer and symptom expression in infected citrus (study 3). We are also investigating how transmission efficiency varies depending on the duration of the inoculation access period (study 4), and if a systemic insecticide can prevent transmission (study 5). We finished study 1 and are close to conclusion of studies 2 and 3. Studies 4 and 5 are being set up and will require another year for completion. In this quarterly report we show results of experiments related to study 1 (see previous reports for partial results of studies 2 and 3).

In study 1, we compared acquisition efficiency of CLas by different ACP nymphal stadia (1st, 2nd, 3rd, 4th and 5th instars) and adults (1 wk old). Insects of each age group were allowed a 2-day acquisition access period (AAP) on CLas-infected plantas, then kept on citrus healthy plants for 19 days and tested by real-time (RT) PCR. The experiment was repeated three times with different source plants. Mean acquisition efficiencies by 1st, 2nd, 3rd, 4th and 5th instars, as measured by the proportion of infective psyllids by RT-PCR, 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. In a second experiment, we compared CLas acquisition efficiency by 3rd-instar nymphs and adults in relation to duration of the AAP (1.5, 6, 12, 24, 48 or 96 h). Almost 30% of the nymphs and adults were PCR positive for CLas when submitted to the shortest AAP tested, suggesting that the minimum AAP required for acquisition is <1.5 h. Acquisition efficiency by nymphs increased at a logarithmic rate in relation to AAP duration, reaching an average of 91% with 24-h AAP and stabilizing around this level with longer AAPs. In contrast, acquisition efficiency of CLas by adults increased linearly with AAP duration, reaching the maximum level (93%) only after an AAP of 96 h. Overall, the results show that both nymphs and adults can efficiently acquire the pathogen if allowed to feed for 1 and 4 days, respectively, on young shoots of infected plants.

In a third experiment, we used the Electrical Penetration Graph (EPG) technique to determine stylet penetration activities and probing periods required for pathogen acquisition and inoculation in citrus. Healthy ACP adults were connected to the EPG system and placed on CLas-infected plants for different probing periods. Probes were artificially terminated by pulling the insects out of the plant after the following stylet penetration periods (treatments; n=30 insects per treatment): I) 20 min in waveform C (pathway phase through epidermis and parenchyma); II) waveform C + 30 s in waveform D (first contact with phloem tissue); III) waveforms C+D + 90 s in waveform E1 (penetration and possibly salivation in the phloem sieve elements); and IV) waveforms C+D+E1+ 1 h in E2 (phloem sap ingestion). Following each probing treatment, the insects were kept on healthy seedlings for 21 days and then tested by RT-PCR for CLas infectivity. We observed infective psyllids (5 out of 30) only when waveform E2 was performed (treat. IV), indicating that CLas acquisition by ACP occurs only after its stylets reach the phloem tissue, and sap ingestion (represented by E2) takes place. We hope to obtain additional replicates per treatment to confirm these results. This knowledge is important to analyze the efficacy of some control tatics (e.g. insecticides, host plant resistance and feeding deterrents) aimed to prevent pathogen acquisition by ACP.

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Contract Number NAS ID Lopes-126

Project Duration (years) 3 *Year of Project* 2

Total Funds (current year) $63,200.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.