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)

HLB pathogen has a latent period around 10 days in the vector

Proposal Title

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

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
12/9/11	Citrus Research and Development Foundation	Vector Management

REPORT UPDATE (500 words; summarize your accomplishments)

This project consists of 5 studies about factors that influence acquisition and inoculation of Candidatus Liberibacter asiaticus (LAS) by th Asian citrus psyllid (ACP), Diaphorina citri. This knowledge is basic to understand the pathogen transmission process and determine more efficient management strategies to manage Huanglongbing (HLB). The results of the first 3 studies on effects of vector development stadia and duration of acquisition access period (AAP) (study 1), leaf age (study 2), pathogen titer and symptom expression in infected citrus (study 3), were shown in the earlier reports of this project. The project is progressing as planned. Studies 1-3 are concluded and most experiments related to studies 4 and 5 were already set up.

This report describes partial results of study 4, in which we are investigating the relationship between duration of the inoculation access period (IAP) and transmission efficiency of CLAS by ACP. First we set up an experiment to determine when ACP starts inoculating the pathogen after acquiring it from source plants (latent period), which is important to study the effects of insecticides on transmission. Groups of 3rd-instar nymphs and 1-week old adults obtained from our healthy laboratory colony were confined on LAS-infected sweet orange plants for a 48-h acquisition access period at 25oC. Next, 25 insects of each age group were individually and serially transferred to healthy sweet orange seedlings (test plants) for successive inoculations access periods (IAP) of 48 h at 25oC, until 37 days after the beginning of the acquisition. To assess successful inoculations, TaqMan® Real-time PCR detection assays with LAS-specific primers were performed on DNA extracts from test plants 10 months later. First transmission events were observed after 11 and 13 days from beginning of the AAP by 1st instar nymphs and adults, respectively. This experiment was repeated and the first transmission event was observed after 9 days from the beginning of acquisition by nymphs, whereas no transmission was detected at anytime following acquisition by ACP adults; first symptoms in inoculated test plants were observed at 5 months after inoculation.

In a second latency experiment, we used a longer AAP (96 h) of 3rd-instar nymphs and 1-week old adults on LAS-infected plants and subsequently transferred larger numbers of insects to test plants, in order to increase transmission rates and obtain a better estimate of the minimum latent period. Ten insects of each age group were serially transferred to healthy sweet orange seedlings for successive 48-h IAPs at 25oC, until 33 days after beginning of the AAP. The first transmission event occurred at 11 days after the onset of acquisition by nymphs for most groups tested (14 out of 15 inoculated plants were RT-PCR positive and symptomatic). For ACP groups that acquired LAS as adults, first transmission was detected at13 days from beginning of acquisition, but for only 1 out of 15 groups tested. After the first transmission event, transmission by each group of insets was intermittent, following a random or aggregated pattern. Overall, the results indicate that the minimum latent period of LAS in ACP is approximately 10 and 13 days at 25°C, after acquisition by 3rd-instar nymphs and adults, respectively. It also confirms that the pathogen is transmitted more efficiently by nymphs, as shown in study 1.

PI First Name Joao

PI Last Name Lopes

Email jrslopes@usp.br

Phone +55 (19) 3429-4199, ext. 212

Organization University of Sao Paulo/ESALQ

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Total Funds (current year) $63,200.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.