CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD December 2015

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Factors Influencing the transmission of the huanglongbing (greening) pathogen by the Asian citrus psyllid and methods for interrupting the transmission process

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
2/1/16	Citrus Research and Development Foundation	Vector Management

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

1) As in previous years, ACP egg lays decreased substantially in cooler weather resulting in fewer embryos for injection, a fewer adults to maintain the colony.

2) During this quarter 524 Asian citrus psyllid (ACP) control uninjected eggs were set-up yielding a 36.3% nymphal hatch rate, resulting in an approx. increase of 11% above the previous quarter. 1,314 eggs were micro-injected with transformation vector/helper plasmids having a 4.2% hatch frequency, approx. 1% greater the previous quarter. Of the 55 newly hatched nymphs from injected eggs, 22 survived to adulthood and were backcrossed to wild (uninjected) ACP adults. Though none of the 63 G1 offspring exhibited DsRed fluorescence, a significant improvement in ACP viability post-injection from nymph to adulthood was achieved. Further improvement may be possible after completion of the environmental chamber retro-fit for temperature, humidity and lighting control.

3) To further improve embryo injections, nymphal hatching and survival to adulthood tests have been initiated to determine optimal heat shock temperature and duration. 722 eggs were collected and placed on tape, but not injected. After 18 hours 505 were heat shocked at 37°C for 45 min of which 20.2% hatched as nymphs, while 217 eggs that were not heat shocked (maintained at 25°C) had a hatch rate of 18.4% indicating that typical heat shock conditions did not have a negative effect on nymphal hatching..

4) Reverse-transcriptase PCR (RT-PCR) was continued to test DsRed marker expression in ACP injected with vector plasmid (containing the IE1-DsRed marker) to determine if the IE1 baculovirus promoter functions normally in this species (as it does in caribfly, A. suspensa). For the A. suspensa controls and ACP samples, 400 embryos from each species were injected with pBac[IE1hr5-DsRed] vector plasmid and incubated for 24 hr before RNA extraction, and subsequent RT for cDNA production. cDNA from each sample and uninjected ACP and a water control were then subjected to PCR using DsRed primers which yielded positive DsRed expression in both A. suspensa and ACP, though approximately 2-fold higher in A. suspensa. This indicates IE1 promoter does function in ACP, though possibly less functional compared to A. suspensa. Tests will be continued to verify and quantify this activity, and similar tests will be performed to quantify piggyBac transposase expression in ACP.

PI First Name Kirsten

PI Last Name Pelz-Stelinski

Email pelzstelinski@ufl.edu

Phone 863-956-8666

Organization University of Florida

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Form PR-12 Quarterly Reports **SUBMIT REPORT**

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.