CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD June 2014

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Identification of Bacillus thuringiensis endotoxins active against Adult Asian Citrus Psyllid

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
7/3/14	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.)

The objective of the current project is the identification of a Bacillus thuringiensis (Bt) crystal toxin with basal toxicity against Asian Citrus Psyllid (ACP) and enhancement of this toxin by addition of an ACP gut binding peptide. A phage display library will be screened to identify peptides that bind to the gut of the ACP. Addition of the gut binding peptide to the Bt toxin will increase toxin binding and associated toxicity against the ACP.

During the reporting period, the proteolytic profile of 15 Bt isolates provided by Dr. Michael Blackburn, USDA Maryland was characterized at Iowa State University. Briefly, Bt toxins were solubilized using sodium carbonate pH 10.5, 10mM DTT, lysozyme 200 micrograms per milliliter for 3 to 16 hours at 37°C, according to the solubility of each strain. The samples were dialyzed for 21 hours with three buffer exchanges against 50 mM Tris-Cl pH 8.5. Ten micrograms of Bt toxins were incubated with bovine trypsin at a final concentration of 10% of the toxin concentration at 37 °C for 1 or 2 hours for determining the proteolytic profile. Finally a 1 hour activation of a high protein concentration sample was performed to obtain sufficient toxin for toxicity assays. Removal of trypsin was carried out using benzamidine sepharose incubating the toxin sample at room temperature for 30 minutes with removal of the sepharose by centrifugation. The samples were verified by SDS-PAGE and the protein concentration was quantified by Bradford assay. Samples were shipped to USDA ARS Florida for testing by Dr. David Hall .

ACP bioassays of five of the Bt isolates have been conducted at USDA ARS Florida. However, the psyllid mortality rates exposed to diet alone confounded conclusions from these assays. A series of experiments were conducted to address the high mortality in the negative control treatment. The psyllid survival rates have now improved, apparently because of seasonal effects on psyllid acceptance of the artificial diet, with low survival in the winter months. Additional challenges associated with the psyllid bioassays were 1) the amounts of Bt isolates provided only allowed for 4 replications of each dose; Additional replications may minimize the impact of variation between replicates. 2) The diet typically includes a yellow-green food coloring dye, but this dye precipitated when the Bt samples were added. The dye is now omitted from the diet and something green placed behind the diet sachet to promote feeding. 3) Bt toxins activated at Iowa State University are supplied in buffer (Tris-HCl pH 8.8). Assays are underway with this buffer to test for toxicity against psyllids. An appropriate amount of buffer will be added to control diet for future assays.

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Project Number 711

Project Duration (years) 3 *Year of Project* 1

Total Funds (current year) $169,363.00

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