CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD December 2013

(●) *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)
2/10/14	Citrus Research and Development Foundation	Unclassified

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

The goal of the project is to identify a Bacillus thuringiensis (Bt) toxin with toxicity against Asian Citrus Psyllid (ACP) and to improve its toxicity by genetic modification with a psyllid gut binding peptide. The peptide will be identified by screening a phage display library for peptides that bind to the gut of the ACP. Addition of the selected peptide to the identified Bt toxin will result in significant enhancement of toxicity of the modified toxin relative to the wild type toxin toward the ACP.

Durign the current reporting period, the focus was on trypsin activation of the partially purified Bt toxins from selected Bt strains provided by USDA ARS, MD. Trypsin activation of Bt toxins was carried out at Iowa State University. Briefly, Bt toxins were solubilized using sodium carbonate pH 10.5 + 10mM DTT for three hours at 37°C and dialyzed against 50 mM Tris-Cl pH 8.5. Bt toxins were then incubated with bovine trypsin at a final concentration of 10% of the toxin concentration at 37 °C for 1 h. Removal of trypsin was carried out using benzamidine sepharose. The samples were boiled in denaturing SDS sample buffer for 5 min, separated on 10% (wt/vol) SDS/PAGE and stained with Coomassie blue.

Different SDS-PAGE profiles of trypsin-treated Bt toxins were apparent with multiple molecular weight protein bands ranging from ~18 kDa to ~150 kDa. Based on the similarity of the molecular mass of the toxin profiles, eleven Bt strains are classified into the following six groups. These groups are distinct from the five groups reported in the previous update.

Group one: Five Bt strains, each with three activated toxin bands of ~60, ~65 and ~70 kDa.
Group two: Two Bt strains, each with two activated toxin bands of ~60 kDa.
Group three: One Bt strain with one activated toxin band of ~60 and ~65 kDa.
Group four: One Bt strain with one activated toxin band of ~70 and~73 kDa.
Group five: One Bt strain with activated toxin bands of ~70, ~90, and ~150 kDa.
Group six: One Bt strain with activated toxin bands of ~18, ~20, ~22, ~28,~30, ~35, ~49, ~60, and ~70 kDa.

Two milligrams of each of four trypsin-activated Bt toxin samples were sent to Dr. David G. Hall, USDA-ARS for ACP membrane feeding assays for toxicity analysis.

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Project Number 711

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

Total Funds (current year) $169,363.00

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

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