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)

Controlling psyllids with dsRNAs targeting essential genes

Proposal Title

Control of the Asian citrus psyllid, Diaphornina citri Kuwayama with protease inhibitors and RNAi.

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
3/2/11	Citrus Research and Development Foundation	Transgenic/Metagenomic Mediation of Resistance

REPORT UPDATE (500 words; summarize your accomplishments)

This research project is directed towards controlling psyllids using biologically-based control strategies that employ the use of RNAi technology against key biological control pathways, peptide hormones and protein inhibitors that, if expressed in transgenic citrus, would enhance plant resistance to psyllid feeding. Both protein-based and RNAi strategies were tested by feeding psyllids artificial diets. To support the artificial diet assays, we optimized the diet composition by adding an antimicrobial agent to eliminate fungal growth that is introduced by the psyllids during the assay period. Using this approach we identified suitable buffers and optimal diet pH during the feeding period. In separate experiments, Tryspin Modulating Oostatic factor (TMOF), a mosquito decapeptide hormone, and cysteine protease inhibitor (CPI) from the Asian Citrus psayllids that was identified in our laboratory were added to artificial diets on which psyllids were allowed to feed. After 10 days of feeding, 100% mortality was observed in psyllids feeding on diets containing TMOF or CPI, whereas, 40% mortality was found in psyllids feeding on the control diets. CPI fed psyllids caused a significant higher mortality than the controls after 7 days of feeding.

Based on these observations a collaborative project with Dr. Bill Dawson's laboratory (Univ. of Florida, IFAS, CREC, Lake Alfred, FL) was initiated to use a Citrus tristeza virus (CTV) expression vector to produce TMOF and CPI in the in the citrus phloem. Clones containing the sequences of mosquito TMOF and CPI (cathepsin protease inhibitor) (both shown to be effective against psyllids) were provided to Dr. Dawson's laboratory. Once CTV vectors are constructed and used to infect citrus plants, Dr. Dawson's laboratory will provide us with the plants to evaluate the effect on psyllids while feeding on these plants.

In parallel to these studies, we synthesized dsRNA molecules targeting 11 different psyllids essential genes encoding three different classes of proteins (alpha-tubuliln, V-ATPase, and Cathepsins). Initial feeding studies with alpha-tubulin dsRNA and V-ATPase dsRNA caused ~60% psyllids mortality as compared to only ~30% mortality for psyllids fed a control diet containing an equal amount of dsRNA not specific to the psyllid.

Using this information, we have initiated experiments to produce citrus plants that express these dsRNAs in citrus phloem cells. A collaborative project with Dr. Bill Dawson's laboratory (Univ. of Florida, IFAS, CREC, Lake Alfred, FL) was initiated to use a Citrus Tristeza Virus (CTV) expression vector to produce the psyllid dsRNAs in the citrus phloem. Clones containing the sequences of a psyllid Vacuolar ATPase and Cathepsin protease (both shown to be effective dsRNA targets) were provided to Dr. Dawson's laboratory. Once CTV vectors are constructed and used to infect citrus plants, Dr. Dawson's laboratory will provide us the plants to evaluate the effect on psyllids while feeding on these plants. We have also designed expression vectors for the production of transgenic plants expressing the psyllid dsRNAs through Agrobacterium-mediated plant transformation. These will be used to initiate the production of transgenic citrus plants that constitutively produce these dsRNAs in the phloem.

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Organization University of Florida

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

Total Funds (current year) $196,401.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.