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)
12/2/10	Citrus Research and Development Foundation	Transgenic/Metagenomic Mediation of Resistance

REPORT UPDATE (500 words; summarize your accomplishments)

Our 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. During the first year of the grant's period peptides, proteins and RNAi moieties were tested by feeding them to psyllids using artificial diets. The diet was optimized by adding an antimicrobial agent to eliminate fungal growth that is introduced by the psyllids during the assay period and we identified suitable buffers and optimal pH. Tryspin Modulating Oostatic factor (TMOF), a mosquito decapeptide hormone, and cysteine protease inhibitor (CPI) from Diaprepes abbreviatus, the citrus root weevil, were found to be excellent candidates; causing high mortalities when fed to psyllids by artifical diet. Ten psyllids genes representing three gene families of cathepsins (five genes), vacuolar ATPases (four genes), and tubulin (one gene) were targeted and their dsRNA (16 ng/µL) fed to psyllids using artificial diets. Three vacuolar ATPases and three cathepsins (B, L and F) showed significantly higher mortality than the controls.

In the first quarter of the second year period our studies continued to characterize the cause of increased psyllid mortality induced by feeding of Double-stranded RNA (dsRNA) molecules targeting specific psyllid genes. Large scale experiments were conducted to harvest sufficient RNA for Northern blot characterization of the integrity and abundance of specific psyllid mRNAs that were targeted and showed enhanced insect morality. The Northern blot analyses although cumbersome and time consuming, are essential for complementing Q-RT-PCR based analyses of targeted transcript abundance.

To further support and enhance our RNAi research observations using artificial feeding chambers, we developed a detached leaf assay that supports adult and nymph psyllid survival and allows dsRNA uptake into intact citrus leaves on which the psyllid are naturally feeding. Initial results suggest that transcript specific mortality induced by feeding dsRNA to psyllids in artificial diets can be reproduced using the detached citrus leaf assay. The assay was developed to show that low doses of dsRNA circulating within the phloem can shut down key biological genes in psyllids when ingested, and thus support the possibility that RNAi strategies can be developed to control psyllid feeding on citrus and, therefore, control the spread of HLB.

As part of this research a dsRNA virus was also discovered in psyllids and was characterized. This virus is present in natural psyllid populations within Florida, but accumulates to higher levels when the psyllids are maintained in greenhouse colonies. Because it is possible that dsRNA viruses can suppress the RNAi machinery of an insect, we are currently developing dsRNA of virus free psyllid colonies to support future RNAi research in psyllids.

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Organization University of Florida

Contract Number 00072711

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

Total Funds (current year) $196,401.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.