Florida Citrus Advanced Technology Program

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

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	Psyllid	1/5/10

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

The effect of peptides, Cysteine Protease Inhibitor and dsRNA targeting essential genes on Asian citrus psyllids survival

TITLE and CONTACT INFORMATION

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

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Organization University of Florida-IFAS, FMEL	*Total Direct Funds (current year)* $196,401

REPORT UPDATE (650 words; provide details about your headline)

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. TMOF was tested at a concentration of 10 µg/µL and CPI was tested at a concentration of 3 µg/µL. Since psyllids take in very small amount of the artificial diet, in nl quantities, the amounts of the tested proteins are physiological rather than pharmacological. 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. The earliest significant effect of added TMOF to the diet was observed at 4 days of feeding. TMOF caused ~15% mortality whereas the mortality of the control group was less than 5%. CPI fed psyllids caused a significant higher mortality than the controls after 7 days of feeding. CPI was tested at lower concentrations that were used for TMOF, because of limited availability of the purified protein. Because of these results we plan to purify more CPI for subsequent testing. In the fourth quarter of the grant period we plan to expand this work and study dose effect and optimal concentrations, as well as, potential for synergistic effects when both TMOF and CPI are fed together to psyllids in the same diet. Continued success of this strategy will allow us to develop transgenic citrus expressing these proteins in the phloem making citrus resistant to adult and nymph psyllids. A third polypeptide was also tested at concentrations similar to TMOF, this polypeptide prevented psyllids from successful eating the artificial diet causing starvation and death. We plan to continue these studies and further characterize the polypeptide and feed it in concert with TMOF and CPI to be able to find out the combined effect on the Asian citrus psyllids.

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). The DNA encoding a fragment of each of the 11 genes was synthesized and cloned into appropriate vectors. Primers used for dsRNA synthesis using the Megascript kit from Invitrogen was used to produce double stranded RNA (dsRNA) for RNA inhibition (RNAi) studies. We produced dsRNA for two alpha-tubulins and two V-ATPases. 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. Influence of dsRNA on cognate psyllid transcripts will be tested in psyllids that remain alive at 8 days.

In summary we:

1. Prepared dsRNA molecules against alpha-tubulin and V-ATPase and fed them to Asian citrus psyllids showing that they are very effective causing high mortality.
2. Showed that physiological amounts of TMOF, a new polypeptide that was recently discovered in our lab and CPI that were fed to psyllids by an artificial diet caused 100% mortality.