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	7/22/10

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 psyllids feeding. DIET: Both protein-based and RNAi strategies were tested by feeding psyllids artificial diets. Both protein-based and RNAi strategies were tested using artificial diets on which pysllids were fed. Psyllids in nature, feed on phloem content of citrus and its relatives. Thus, psyllids do not tolerate many alterations to diet composition that is drastically different than the phloem content. Addition of high concentrations of proteins or single stranded and double stranded RNA (ssRNA and dsRNA) reduces psyllids survival. Therefore, we determined the acceptable concentrations of each molecule and cofactor that was added including a suitable buffer to allow continuous feeding and maintenance of a physiological pH that was not detrimental to psyllids. We also identified an antimicrobial agent that was added to the diet and prevented fungal growth but did not harm the psyllids or their associated and obligate symbiotic microflora. Prior to the identification of the antifungal agent, fungal contamination of the diet caused unacceptable high level of psyllids mortality because the fungus is carried by the psyllids and can enter the diet through the psyllids feeding process. Control experiments showed that addition of dsRNA molecules, that did not target psyllids transcripts, at up to ~16 ng/uL improved psyllids performance, but above this concentration, the non-specific dsRNA would reduce psyllids survival. Therefore, comparisons of efficacy of specific psyllids gene targeting dsRNA were done with dsRNA that did not target psyllids genes. PROTEIN: In separate experiments, mosquito peptide hormone, TMOF, and Diaprepes abbreviates (citrus root weevil) cysteine protease inhibitor (CPI) were added to an artificial diet that was fed to psyllids. TMOF and CPI were tested at concentrations of 10 µg/µL and 3 µg/µL, respectively. After 10 days of feeding, all the psyllids that were fed diets containing either TMOF or CPI died, whereas only 40% mortality was observed in psyllids that fed on the control diets. TMOF caused 15% mortality after 4 days of feeding as compared with less than 5% mortality in the control group. Psyllids that were fed CPI did not show significantly higher mortality than the controls until after 7 days of feeding, because CPI was tested at less than ½ the concentration that was used for the TMOF because of limited availability. During the second year of the grant's period more CPI will be synthesized and purified to study dose effect and optimal concentration, as well as, potential for synergistic effects when both proteins are present within the same diet. RNAi: 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. The earliest effects were observed at ~4 days after feeding and feeding continued until day 10. . We have identified two of the best target genes to date as being a Cathepsin (CF2) and a Vacuolar ATPase (Vatpase-3). When specific dsRNA molecules to each are fed separately to adult psyllids we see a doubling in psyllid mortality over control non-specific dsRNA molecules at 6 to 7 days with 48 ng/uL of dsRNA in the diet. However, when they are supplied together we see similar mortality rates at 1/10th the concentration. At the lowest concentrations tested (3 ng/uL) we see mortality that appears to be non-sequence specific. However,sequences targeting CF2 and Vatpase-3 show a much more rapid increase in mortality as concentrations exceed 6 ng/uL. These results suggest that antagonistic effects of ingestion of low concentrations of any dsRNA may provide limited benefit and that by combining this effect with psyllid specific dsRNAs significant control can be realized. Based on these results, the production of transgenic plants expressing a chimeric CF2/Vatpase-3 dsRNA producing gene is being initiated.