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	6/8/09

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

Synthesis and cloning of RNAi and Protease Inhibitors

TITLE and CONTACT INFORMATION

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

Principal Investigator Dov Borovsky	*PI Last Name* Borovsky
Email dobo@ufl.edu	*FDACS Contract Number* 00072711
Phone 772-778-7200 Ext 141	*Project Duration (years)* 3 *Year of Project* 1
Organization University of Florida- IFAS, FMEL	*Total Direct Funds (current year)* $196,401

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

Our research project is directed towards controlling adult and nymph psyllids by using biorational and genetically engineered factors and Long Hairpin (lh)RNA. We propose to develop a novel TMOF and RNAi technologies that target proteases and other important physiological events in the gut of psyllids. In order to achieve this goal, we prepared EST libraries to find out the important digestive enzymes that psyllids use to digest their food. The most abundant digestive enzymes found in the EST libraries are cathepsins F, B and L. Our EST library also identified several other key molecules that are essential for adult and nymph growth and development like alpha-Tubulin important in cells growth and division, and vacuolar ATPAse that is important in water and ion transport through the gut. Using the sequences that are available to us from the EST libraries seventeen DNA molecules of 300 nt flanked at the 5' and 3' by T7 promoters were synthesized and cloned into pUC57 plasmid. We are at the moment sequencing the plasmids and using restriction enzyme analysis to show that the sequences are correct. Once the sequences are confirmed we will use RNA polymerase to convert the DNA into dsRNA. In parallel, our collaborators at the USDA and at the University of Florida in Ft Pierce were successful in maintaining a healthy psyllid colony for feeding tests. These insects can be artificially fed through a membrane with various nutrients and can be kept alive for up to 10 days allowing us to test the lethal effect of our dsRNAs and protease inhibitors on adult and nymph psyllids.

The second thrust of AIM1 in our funded project is to produce proteins and peptide hormones (for example, TMOF) that can be used to inhibit food digestion in adults and nymphs. Because our EST libraries indicate that the major digestive enzymes in the gut are cathepsin like enzymes, we have cloned and expressed a specific cystein protease inhibitor, that we have discovered in the gut of the citrus weevil, Diaprepes abbreviatus. The inhibitor was expressed in E. coli and bacterial cells were grown for 24 h at room temperature to prevent non-specific precipitation of the recombinant protein in the bacterial inclusion bodies. Cells were broken and supernatants were purified by Nickel affinity chromatography. About 3.0 mg of the inhibitor were harvested and aliquots characterized by SDS polyacrylamide gel electrophoresis. The protein migrated as a single band on the gel indicating that the protein was at least 90% pure. Some of the protein was sent to the University of Florida Biotechnology Institute and mass spectrometry analysis confirmed that the protein is indeed cystein protease inhibitor. The protein was incubated with crude cathepsins gut homogenates from psyllids and Diaprepes. About 200 to 5000 ng of the recombinant protease inhibitor completely stopped cathepsin activity in the crude extracts for hours. These results indicate that we can now start using our artificial feeder to test the effect of the protease inhibitor on psyllids growth and development.

In summary we :
1. Cloned potential dsRNA molecules that are essential for cell growth, water and ions balance and digestion.
2. Bio-engineered E. coli with a cystein protease inhibitor, stimulated the bacteria to produce the protein, purified and characterized the protein.
3. Developed a feeding apparatus that allows us to test bio-engineered dsRNA molecules and protease inhibitors.