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	10/14/09

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

Preparation of dsRNA and feeding of TMOF and cysteine protease inhibitor to Asian citrus psyllids

TITLE and CONTACT INFORMATION

Proposal Title Control of the Asian citrus psyllid, Diaphornina 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	*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)

This research project is directed towards controlling psyllids using RNAi technologies, as well as, protease inhibitors and peptide hormones that, if expressed in transgenic citrus, would kill adult and nymph psyllids. We tested this approach by feeding TMOF, a mosquito decapeptide hormone that stops trypsin biosynthesis in adults and larval gut, and a citrus weevil cysteine protease inhibitor (CPI). Each was added separately to an artificial diet on which psyllids were allowed to feed through a membrane. After 10 days of feeding TMOF (10 µg/µL) and CPI (3 µg/µL), all the psyllids that fed on the diet died (100% mortality). In control diets without TMOF or CPI only 40% died. The earliest significant effect on psyllids survival was observed at day 4 after feeding TMOF. The mortality of Psyllids that fed TMOF for 4 days was 15% whereas the mortality of the control group was less than 5%. Psyllids that were fed CPI did not show a significant higher mortality than controls until day 7. However, CPI concentration in the diet is 3.3-fold lower than the concentration that was used for TMOF. Because CPI is produced by genetic engineering of E. coli, its availability is limited, whereas TMOF was commercially synthesized. To continue these studies we are now fermenting large amounts of E. coli in order to purify high amounts of CPI to study the effect of different doses and optimal concentrations of this inhibitor on psyllids. We are cognizant to the possibility that both proteins when fed together may show synergism and will be looking for such phenomenon that was recently discovered in our lab when TMOF and cry toxins were fed together to mosquito larvae.

The ultimate purpose of this project is to develop transgenic citrus plants expressing these proteins. Our results show that this will be feasible after dose response curves will be developed to calculate the minimal amounts of the recombinant proteins that are needed to be circulated in the citrus phloem to effectively control adult and nymph psyllids.

Currently we are synthesizing double-stranded(ds) RNA targeting 11 different psyllid genes encoding three different classes of essential proteins that control cell division, digestion, water and ion balance in psyllids cells. The DNA that encodes fragments of each of the 11 genes was synthesized and cloned into appropriate vectors. Primers for the dsRNA synthesis using the RNA replicator kit (Finnzyme) were synthesized and used to produce dsRNA. After optimizing the procedure, dsRNA of individual genes will be fed to the psyllids using artificial diet and the effect of the dsRNAs on psyllid-mortality will be monitored for 10 days. At different times during the feeding, psyllids will assayed by qPCR or Northern blot analyses for RNA transcripts that are being targeted by the dsRNAs. To enhance psyllids survival on the artificial diet, we optimized the diet composition by incorporating antimicrobial agent to reduce fungal growth in the diet, and identified suitable buffers to support optimal diet and pH balance during the feeding period.

In summary we:
1. Prepared 11 DNA clones that will be converted to dsRNA for feeding experiments
2. Optimized the feeding diets with antimicrobial and fungal agent and balanced the pH.
3. Showed that feeding TMOF or CPI for 10 days causes 100% mortality to psyllids.