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*	Genetics	7/15/10

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

Six EST libraries constructed, assembled, and data mining underway; FISH optimization

TITLE and CONTACT INFORMATION

Proposal Title The citrus psyllid transcriptome and time course differential gene expression in Ca. Liberibacter-infected/free whole psyllids and organs

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Phone 520-621-1402	*Project Duration (years)* 3 *Year of Project* 1
Organization The University of Arizona; University of Florida	*Total Direct Funds (current year)* $164,999 + sub 20,787

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

Six cDNA libraries were constructed from (a) adult/immature psyllids, dissected gut, salivary glands (PSGs) and accessory salivary glands (ASGs). The cDNA synthesis was based on the total amount of RNA: (a) The yield of total RNA for uninfected 1000 guts (PG) was 10.22 ug at a concentration 511 ng/ul in 20 ul. ESTs were trimmed and assembled, organized, and annotated using PAVE. NCBI nr db reveals short read matches to psyllid primary endosymbiont, while short and long EST reads were annotated using Uniprot. Prelim conclusion: endosymbiont nr db matches primarily, the primary sym Carsonella ruddii; ESTs encode psyllid proteins; (b) For psyllid Ca. Liberi-infected adults (PI), total RNA was obtained @11.44 ug at a concentration 572 ng/ul in a total volume of 20 ul) from tube 'P-INF' 8/12/2009: infected) for the library construction. Cataloging genes/proteins is underway for six libraries. We have made good progress optimizing the FISH assay on whole psyllids (adults, immatures) and dissected organs to minimize auto fluorescence and maximize signal using a probe for the primary endosymbiont 16S rRNA.

Having sequenced random cDNA clones, assembled ESTs, and initiating data mining toward gene validation using FISH, we will next make libraries for short base read sequencing (RNAseq) to quantify expression in adults, immature instars, and eventually organs. We will carry out extensive sequencing for HLB+/- stages, instars, and organs (instead of microarray analysis) because the relative cost of sequencing has declined, as the extent of coverage vs. cost has increased. In this way we can more effectively compare expression levels between adult and immature psyllids, guts and salivary glands.

Based on preliminary mining the pyro-sequencing and Illumina sequencing were highly successful; hits predict psyllid and primary endosymbiont, as well as other prokaryotic genes, including Ca. Liberibacter in infected psyllid colonies. In addition the putative 'uninfected' psyllids gave no Ca. Liberibacter hits, confirming colonies are HLB free, as has been indicated by qPCR results. Further, to date no phytoplasma sequences have been detected. Because studies indicated that 5th instar nymphs might better support Liberibacter accumulation over the adults, we constructed the following EST libraries: LB+/- adult psyllids; HLB+/- adult guts, and whole HLB+/- 4-5th instar psyllids. The rationale is that PSG/ASG transcripts will be present in the whole adult and 4-5th immature instar HLB+/- libraries. The salivary gland libraries will be constructed in Yr 2 for the potato psyllid because it is a more tractable system; once parameters are established to identify the point at which the bacterial titer is highest in these same organs for the Asian citrus psyllid PSG/ASG libraries will be made.

Quantification can be achieved based on the downstream random cDNAs sequenced from HLB+/- adults, given a range of AAPs (0-40 days), compared to ESTs from adult or immature instars born and reared on HLB+/- plants. In this way we will learn how 4-5th immature instars compare to adults as reproductive hosts, and presently we are considering HLB+/- whole immature instars reared on infected plants. This will allow us to quantify gene expression in the various treatments, stages, and organs, while requiring fewer insects and organs (for mRNA) from time-course studies.