CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

COI analysis; FISH primers/revising design; qPCR; SEM

Proposal Title

Gross and fine structure localization of Liberibacter in citrus psyllid Diaphorina citri organs: elucidating the transmission pathway.

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
9/27/10	Citrus Research and Development Foundation	Infection Responses

REPORT UPDATE (500 words; summarize your accomplishments)

Obj. 1.DNA bar coding to establish the identity and diversity in south Florida (Stansly, Brown). new mtCOI primers were designed based on amplicons obtained using general homopteran primers, resulting in increased specificity. PCR primers were used to obtain a ~1200 bp product; sequence analysis is underway.
Obj. 2. qPCR detection of Ca. Liberibacter presence (or absence) in the psyllid colony cohorts over different IAPs has been optimized and analysis is underway for the first two replicates.
Obj 3. Define the gross association of Ca. Liberibacter in thick sections…develop a 'gross anatomical road map' of Ca. Liberibacter accumulation in key organs, tissues, and cells; Obj. 4 Using the resultant 'thick section road map', elucidate at the TEM level the specific organs, tissues and cells where Ca. Liberibacter accumulates in the vector. We have adopted the potato psyllid, Bactericera cockerelli, as a surrogate for the citrus psyllid because we can rear it on tomato easily, and set experimental parameters ('acquisition access', 'inoculation access' etc.) that have the tightest possible definition with regard to timespan of actual feeding and source infectivity at the locus of the stylet. Our rearing system is ramped up to broad, robust capacity, providing for multiple studies by several experimenters in the transmission context simultaneously. Guts of infective and uninfective specimens of all life cycle stages (1st thru 5th instars, metamorphosing adult, unpigmented emerging adult ('tenerals'), and mature adult) have been processed, mounted on stubs, and are being studied under the scanning electron microscope (SEM). Graphic differences occur that correlate directly to host-vector relationships at the histological level. These comparisons assist us with interpretation of cross-sections, and demonstrate the abundance of bacteria in infected adults relative to indigenous flora, as well as the buildup of bacteria during each consecutive stage. The pre-oral organ complex, referred to as the 'oral box', is the 0.1mm3 chamber that houses the convergence of the salivary ducts and esophagus, and the transfer of their fluids to the stylets, which in turn, penetrate the plant for feeding. The potential for bacteria to access the stylets, through this box, during egestion events, is very high and cannot be overlooked. Along these lines, it is known that in Psyllidae, the unused length of the larval stylets is externally wound into a groove around the face, but the unused length of the adult stylets is held inside an internal sac (Pollard 1970, Ullman and McLean 1986). Our studies of the oral box have shown that the internalization process retains the cuticular barrier between stylets and blood. The basal sections inside the oral box, however, may undergo modification during metamorphosis that can admit bacteria, and must be given scrutiny as well. 10-15 infective and uninfective adults + source plant material were collocated to the same paraffin block face in four different runs. 4-micron thick sections were cut from these blocks and mounted in alternation on slides and stubs for, respectively, the light microscope and the SEM. Slides were treated with 'in-situ hybridization' techniques that attach a visible gold/silver label to bacteria, allowing them to be localized inside organs, and directly compared to sections magnified under the SEM. These methods give consistent label in the gut, as well as a more general distribution in the abdomen. Strong signal also occurs in the spaces between salivary gland cells and inside the oral box. These localizations are best pursued further under the transmission electron microscope (TEM). Strong signal occurs in new apical growth ('flush') of infected source plants, but in sharply delimited patches, or 'compartments'.

PI First Name Judith

PI Last Name Brown

Email jbrown@ag.arizona.edu

Phone 520-621-1402

Organization The University of Arizona

Contract Number 34

Project Duration (years) 3 *Year of Project* 2

Total Funds (current year) $119,998.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.