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)

Publications; FISH; oral box; transmission pathway

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)
4/21/12	Citrus Research and Development Foundation	Infection Responses

REPORT UPDATE (500 words; summarize your accomplishments)

The final step in completing the general transmission pathway is determination of the origin of Lib individuals that are able to access the stylets (adult, larval, or both) for reinoculation. Once acquired by adult PoP, the potential for dispersal of Lib by flight relies on its ability to exit the adult, or pass transovarially to the next generation larvae. Exit from either is, potentially, multidirectional. Our methodology is therefore scenario driven.

Scenario #1) [Inoculum comes from primarily from larvae]. Our, and other lab's, feeding experiments show that adults can transmit Lib during feeding, but our TEM shows no transepithelial, nor transcuticular, movement (n hot=20, n cold = 10), which would explain the origin of their midgut biofilms. If transmission by the adult is very inefficient, it must be transovarially transmitted to the next generation larvae after flight. SEM of all instars (n=333, 195) shows that 3rd and older larvae have biofilms on the midgut exterior also, but they have yet to be examined by TEM. It may be that larvae are the primary source of inoculum under field conditions. FISH of dissected guts assisted in identification of Lib, but does not distinguish between internal and external bacteria as needed for this scenario (pub. #1).
#2) [Transepithelial movement to the adult midgut exterior may occur at point loci missed in our sampling.] FISH with thick paraffin sections of infra-hour time course AAP is required to see if Lib leaks thru the epithelium at point loci to establish blood-side biofilms.
#3) [Access to the adult midgut exterior by chitinolysis through foregut/hindgut intima.] Alternatively, Lib may chitinolyse through weak points in the intima, and sprawl to the midgut exterior.
#4) [Adult midgut biofilms as a source of inoculum.] Although the origin of adult midgut biofilms is unknown, we demonstrated that they sprawl anteriorly, proliferate in the oral region, burrow into the salivary glands, and reach their core. We have not found them inside the ducts yet, but this anatomy may get clogged or disabled by the biofilm's intrusive movements. Lib are ca. half the diameter of the duct lumen. Alternatively, Lib may also access the mouthparts by chitinolysis through the oral region's endoskeleton (purported for sharpshooters, Brlansky et al. 1983). Our study of the oral region (pubs #2, 3) elucidated its anatomy, but did not show any compromises of cuticle with Lib exiting through them. Views (n = 5) of the oral lumina, available in our TEM libraries, do not show fastidious bacteria, as occurs with sharpshooters (Alves et al. 2008). Our time-course experiments are underway, and when minimum AAP for adults is determined, FISH and colloidal gold may reveal where leakage into the interior occurs.

Publications. We are preparing a third manuscript on the topic of: Stylet biogenesis and installation into their functional positions during the pharate adult stage of the potato psyllid, Bactericera cockerelli (Sulc). J.M. Cicero and J.K. Brown.

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SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.