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)

Working model- bacterial transmission; AAP/IAP time course

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)
10/3/11	Citrus Research and Development Foundation	Infection Responses

REPORT UPDATE (500 words; summarize your accomplishments)

1. mtCOI haplotyping. More samples are being received for mtCOI haplotyping from the R. Lee lab (USDA, CA) to explore baseline diversities in different locations, in an attempt to relate populations from the US and elsewhere in the Western Hemisphere to haplotypes from a primary region of endemism. All sequences in hand have been edited.

2. Time-course and transmission studies continue bioassay to assess transmission frequency in relation to Rt-PCR detection of Ca. Liberibacter solanacearum (AZ) and in Ca. L. asiaticus (FL) in individual psyllids reared on infected plant material. Results using dot blot hybridization were found to be less sensitive than RT-PCR but were generally comparable with a few exceptions; nonetheless we will continue the work using qPCR even though it is the more expensive method, results are robust. Five psyllids reared on infected tomato, and given a range of inoculation access feeds on tomato seedlings from 30 min, and 1,2,4,8,12,and 24 hr transmitted Ca. L. solanacearum 5-20%, 35, 25-30,70, 80, 90, and 95% of the time (20 plants per rep). Plants were assayed using RT-PCR and were scored for symptom development. Only the former data were used to calculate transmission frequencies because psyllid feeding causes aberrations in symptom phenotype that disallow Liberibacter-incitedsymptoms to be differentiated consistently. This above result was corroborated in two replicated studies and a third replicate is underway. Results suggest that once acquired psyllids transmit at relatively high frequency. The goal is to select individuals for light microscopy, and SEM-TEM ultrastructural observations to pin point localization following a range of different AAP and IAPs. We initiated two AAP time points, starting with 8 and 24 hours using a 24 hr IAP.

IAP studies with ACP have proven too difficult owing to the apparent uneven distribution of bacteria in plants, which confounds reliable detection and transmission bioassay to further the localization effort. The goal to produce time course feeding populations for both ACP and potato psyllid is overly ambitious given the problems experienced obtaining ACPs with consistent Liberibacter titer, despite season or host. Even so, extrapolations from the more tractable potato psyllid study system, (an approach approved by the project Director) will clearly help clarify AAP and IAP time-points in relation to organs that are key to the transmission pathway.

3. As noted we are using the potato psyllid as a surrogate for the Asian citrus psyllid since they are closely related, both have digestive systems so similar that we can't find any differences using the methods and instruments available to us (Cicero et al 2010), and, thirdly, the potato psyllid is easy to rear on tomato in our Arizona laboratory. Thanks to timely publications (Cicero et al 2010, Ammar et al 2011a, b), collaborative efforts, and FPRAC quarterly/annual reporting by the teams involved, the general motif for the transmission cycle of Ca. Liberibacter has been worked out in the potato psyllid. We are in publication mode now. Further, we are now developing the toolbox to answer more detailed questions, such as the infra-instar changes in bacterial titers, and the role of the pharate instar in the movement of bacteria from the midgut, where the anatomically operational beginning of the cycle occurs, to the stylets, where the cycle finishes. We are also moving to apply the methods, used for the above breakthrough, on the Asian citrus psyllid as a check to be sure no significant differences occur in that species.

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Organization The University of Arizona

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SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.