Citrus Research and Development Foundation, Inc.

MEMORANDUM

TO: nuPsyllid Management
FROM: Tom Turpen, Project Director
DATE: June 30, 2013
RE: Quarterly Progress Report

Rear and Release Psyllids as Biological Control Agents – An Economical and Feasible Mid-Term Solution For Huanglongbing (HLB) Disease Of Citrus

Project Mission and Organization

The purpose of this NIFA-CAPS is to create attractive options for management of HLB by replacing the wild type insect vector (ACP) with a population that is unable to transmit the bacterial causative agent (CLas). Achieving this outcome will require progress in the following three areas of emphasis – An *Effector Mechanism*, A *Driver System*, and *Diffusion*. The current conditions threatening citrus production nationally require our key personnel to work concurrently on parallel technical plans and to accelerate the leading alternatives based on assessments by our team leaders, advisors and management. These assessments have already begun and will be focused on technical feasibility and of the probability of having an impact on disease control.

TECHNICAL PROGRESS

Effector Mechanism

Initial assessments have not identified the required variation in CLas transmission to occur naturally in ACP populations. However the prospects for engineering a mechanism to achieve the desired phenotype are under active investigation. The effector is the content of the phenotypic change we aim to introduce.

- Sequence and bioinformatic analysis of 129,000 transcripts from from CLas-infected and uninfected ACP nymphs, adult whole bodies, guts, and salivary glands yielded 18 psyllid-encoded genes and 8 CLas-encoded genes that may function as candidate effectors if their activity is perturbed. Numerous additional targets have been identified by gut and salivary gland proteome analysis and through direct physical interaction in the yeast two-hybrid system. Three cDNA libraries have been completed for ACP uninfected gut, uninfected salivary gland, and CLas-infected ACP guts. Candidate effectors are being analyzed through artificial feeding of RNAi.

- From a large expression library screen, a set of 5 single chain antibody fragments (scFv) were identified and shown to bind to specific surface antigens of CLas. Purification of the scFv antibodies are in progress. Construction of a fluorescent tagged peptide library was initiated to identify candidate effectors in a binding inhibition assay using ACP alimentary canal membrane preparations. A plant-based screening protocol was developed for monitoring psyllid transmission that allows full ACP life-stage development in excised plant flush. After infected ACP populations are exposed to dsRNAs that target various ACP genes by feeding *in planta*, the survivors were found to be free of CLas.

Driver System

A new trait will not spread efficiently upon release within an existing population without a genetic bias of some kind. The driver is the medium of spread of the introduced phenotype---lack of CLas transmission. The drivers under investigation are viral, endosymbiont and chromosomal.

- Three viruses of ACP were discovered by deep sequence analysis of small RNA libraries from diverse ACP collections. A virus-free ACP colony was established for biological assays to evaluate these ACP pathogens as potential drivers.
- *Wolbachia* was found to be present in all collections of ACP from 20 different Florida sites with infection frequencies of 86-96%. Two isofemale lines were confirmed to be free of *Wolbachia.* Additionally, antibiotic treatments are being used to clear ACP of *Wolbachia* infection. Two virulent strains of *Wolbachia* have been selected for introduction into *Wolbachia*-cleared psyllid cultures via direct hemolymph transfer or cell culture transfection by microinjection.
- Chromosomal drivers were investigated in the model genetic system *Drosophila* where an invasive system modeled on the naturally occurring *Medea* element has been established. A feature that may be required for release of transgenic insects for some applications is the ability to test the release in the field at a threshold below that required for population replacement. Transgenes would spread to fixation under high release threshold conditions and would be lost under low release threshold conditions. This concept was confirmed in a synthetic system targeting a known haplolethal gene.

Diffusion

Once a nuPsyllid population is developed, its successful use will depend on series of factors based on the overall phenotype and fitness of the population in the environment and most importantly, will depend on human adoption, including the behavior of regulatory agencies, growers and consumers. All of these attributes must be modeled accurately for a nuPsyllid release to be used effectively. As for any other innovation, diffusion is the rate of change. Several aspects of the technical and communication plan can be addressed most effectively only when an actual candidate nuPsyllid is available for release. The ability to rear, release and monitor psyllids has been initiated and are of immediate use in HLB disease management applications outside of this proposal.

- Scented lures for trapping ACP are in field trials in Southern California. It appears that ACP has a similar market behavior to humans, developing a preference for what they were raised on.

SUMMARY

All 8 nuPsyllid Team Leaders and all 4 Scientific and Regulatory Advisors met in person on April 11 and 12th in Riverside, CA at a first Annual Meeting together with MaryLou Polek CO-PD and Tom Turpen, PD. The crux of the technical challenge is to create a driver system that will function as predicted. There is more optimism that effector mechanisms can be developed, although this must continue to be tested concurrently as planned. At this point, we do not underestimate social barriers to adoption of the technology but there is no story to communicate without a nuPsyllid and the story is subtly different depending on the technology. All aspects of the project are proceeding as proposed, reviewed and funded. However, a primary purpose of our meeting was to identify what can be done to maximize the success. Based on the presentations and discussions during and subsequent this Annual Meeting, we will look for extra effort in the following areas:

- Develop and review strategies to achieving psyllid transformation
- Improve our ability to identify the phenotype we are looking for…maximize transmission blockage and minimize fitness loss
- Begin to model the complex system of release and monitoring around hypothetical specifications and explicit assumptions