CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD June 2016

Quarterly Report Final

Proposal Title

High-Throughput Inoculation of Transgenic Citrus for HLB Resistance

Today's Date: 6/29/16

Sponsoring Organization (drop-down): Citrus Research and Development Foundation

Category (drop down): Transgenic/Metagenomic Mediation of Resistance

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

This project (Hall-15-016) is an extension of a project that came to a close last summer (Hall-502). The driving force for this project is the need to evaluate citrus transformed to express proteins that might mitigate HLB, which requires citrus be inoculated with CLas. USDA-ARS-USHRL, Fort Pierce Florida is producing thousands of scion or rootstock plants transformed to express peptides that might mitigate HLB. The more rapidly this germplasm can be evaluated, the sooner we will be able to identify transgenic strategies for controlling HLB. The purpose of this project is to support a high-throughput facility to evaluate transgenic citrus for HLB-resistance. This screening program supports citrus breeding and transformation efforts by Drs. Stover and Bowman. Briefly, individual plants to be inoculated are caged with infected psyllids for two weeks, and then housed for six months in a greenhouse with an open infestation of infected psyllids. Plants are then moved into a psyllid-free greenhouse and evaluated for growth, HLB-symptoms and Las titer, and finally the plants are transplanted to the field where evaluations of resistance continue. CRDF funds for the inoculation program cover the costs associated with establishing and maintaining colonies of infected psyllids; equipment such as insect cages; PCR supplies for assays on psyllid and plant samples from infected colonies; and two GS-7 USDA technicians. A career technician is assigned ~50% to the program. USDA provides for the program two small air-conditioned greenhouses, two walk-in chambers, and a large conventional greenhouse. Currently 18 individual colonies of infected psyllids are maintained. Some of the individual colonies are maintained on CLas-infected lemon plants while others are maintained on CLas-infected Citron plants.

Update: Two technicians funded by the grant have been fully trained in establishing and maintaining colonies of infected psyllids, conducting qPCR assays on plant and psyllid samples, and running the inoculations. As of June 7, 2016, a total of 8,694 plants have passed through inoculation process. A total of 170,895 psyllids from colonies of CLas-infected ACP have been used in no-choice inoculations. Not included in these counts of inoculated plants and psyllids used in inoculations are many plants inoculated over the past year to assess transmission rates, which has provided insight into the success of our inoculation methods and strategies for increasing success.

As reported in the last progress report and reiterated here, research recently showed that seedling citrus with flush is significantly more prone to contracting the HLB pathogen than seedling citrus without flush: Hall, D. G., U. Albrecht, and K. D. Bowman. 2016. Transmission rates of 'Ca. Liberibacter asiaticus' by Asian citrus psyllid are enhanced by the presence and developmental stage of citrus flush. J. Econ. Entomol. 109: 558-563. doi: 10.1093/jee/tow009. Therefore, the program has been changed to ensure that plants to be inoculated have flush.

Current research indicates that the no-choice inoculation step used in our program is successful an average of 79% of the time when approximately 70% of ACP placed on a plant test positive for CLas (Ct <36) and have CLas titers of around CT=26 to 29 (success contingent on flush being present on a plant).

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Organization USDA-ARS

Project Number Hall-15-016

Project Duration (years) 3 *Year of Project* 1

Total Funds (current year) $125,000

Form PR-12 Quarterly Reports SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.