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)

A novel method for efficient inoculation of trees with the HLB

Proposal Title

A novel method for efficient inoculation of trees with the HLB bacterium

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
8/1/14	Citrus Research and Development Foundation	Culture, Molecular Biology, Koch's Postulates

REPORT UPDATE (500 words; summarize your accomplishments)

This project aims in developing a method for efficient inoculation of plants with HLB that could be an alternative to currently used grafting and psyllid-mediated inoculation. We chose to use a Pulse Micro Dose Injection System (PMDIS) for injecting a suspension prepared from tissues collected from citrus trees infected with HLB into leaves and stems of receptor citrus seedlings with a purpose to transmit the bacterium and initiate infection in the latter plants. Since the HLB bacterium is distributed sporadically throughout the infected trees and is present in low quantities, we tested different types of tissue (stems, leaves, seed coats of infected citrus plants as well as tissues of HLB-infected psyllids) as resources of the HLB bacteria for preparation of the inoculum along with testing different extraction buffers for preparation of the bacterial suspension. While doing PMDIS-mediated inoculations, we tested different parameters of injection. We included plants of many species into our experiments: citrus (sweet orange, grapefruit, Citrus macrophylla, Mexican lime, and others) as well as tobacco, periwinkle, papaya. The outcomes of inoculations were evaluated by observation of inoculated plants for symptoms development along with PCR tests with HLB bacterium-specific primers. We have done a large number of inoculations in which we tested different conditions. However, at this point, this new approach was not very successful. Although some infections of citrus plants using PMDIS were achieved, infection rates were far less than those seen upon graft-inoculation of plants with HLB-containing tissue. In order to improve the inoculation technique, we contacted Dr. Carlos F. Gonzalez, Professor at the Center for Phage Technology, Faculty of Genetics, Department of Plant Pathology and Microbiology,Texas A&M University who uses a similar injection system for injection of bacteriophage into grape vines as a part of phage therapy for control of Pierce's disease. A scientist from my lab who has worked on this project visited Gonzalez's lab and participated in injection system-based inoculations in order to learn the protocol that Dr. Gonzalez's lab uses. He also brought back some new nozzles that Dr. Gonzalez uses with his injection device. We have tested those nozzles to see if they would increase the efficiency of our inoculation procedure. Although the funding for this project already ended, we will continue our work. We have a few sets of plants from latests inoculation tests that we maintain in our greenhouse. We will monitor these plants in order to see if any of the changes that we have recently done to improve the procedure have worked. We believe that the key to success in this type of inoculations is a concentrated inoculum containing viable bacteria. Once Ca. L. asiaticus culture becomes available, we will use it with our injection device. The bacterium culture will provide much better inoculum to be tested using this approach. In the meantime, we are testing our injection system with Liberibacter crescens culture.

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Organization University of Florida

Contract Number

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

Total Funds (current year) $94,757.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.