CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL PROGRESS REPORT FORM: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

▶**PROJECT QUARTER END** June 2020 ○ *Quarterly Report* ◉ *Final*

Proposal Title

Use of RNAi delivered by Citrus Tristeza Virus Viral Vector to control the Asian Citrus Psyllid

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
09/01/2020	Citrus Research and Development Foundation	ACP Vector

ABSTRACT (Executive Summary-public report-do not disclose proprietary information or intellectual property)

The purpose of this project was to evaluate the use of Citrus Tristeza Virus viral vectors (CTVvv) containing promising RNAi constructs against the Asian citrus psyllid (ACP) in a small scale field trial in an attempt to duplicate the results obtained in growth chamber experiments. The goals of the project were to:

1. Determine if selected target sequences are effective in controlling ACP
2. Determine the effectiveness of the CTVvv as a delivery method of RNAi
3. Determine the effect of CTVvv+RNAi on CLas
4. Determine the effect of CTVvv+RNAi on the spread of HLB.

The field trial was established 8/23/2017 in a randomized complete block design with 4 different CTVvv-RNAi constructs targeting different ACP genes . At periodic intervals in 2018, 2019 and 2020, adult ACP (from a CLas infected colony) were caged on selected branches of the test trees. ACP survival, ACP reproduction, and citrus greening infection were assessed at 14 and 28 at each date and compared to trees with a CTVvv construct containing an antimicrobial peptide and to untreated control trees. In addition to the introduced caged ACP, tree branches that were naturally infested with wild ACP were also caged and subjected to the same assessments for ACP mortality and reproduction. In addition to the ACP and CLas assays, the status of the CTVvv+RNAi contructs was assayed at each assay date with respect to the presence of CTV in the trees and to determine if the CTVvv retained the RNAi insert or if it had reverted to a wild type CTV isolate (i.e. was the insert stable withing the vector).

With the exception of the initial sample date in April-2018, there were no differences in ACP mortality and reproduction rates for any of the RNAi constructs compared to the untreated controls. Similarly, there were no differences in CLas infection rates for any of the RNAi constructs compared to the untreated and controls. After 3 years in the field, essentially 100% of the trees were infected with citrus greening (under extreme challenge pressure). Vector stability appeared to be an issue for most of the RNAi constructs. By the end of the experiment, most of the CTVVvv+RNAi constructs had thrown out the gene target insert. The one contruct that did show some activity early on, was also apparently the most stable construct. The lack of contruct stability did not appear to be seasonal.

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

% Completion of Objectives (FDACS requirement) 100.00%