CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶PROJECT QUARTER END January 2025 ● *Quarterly Report* ○ *Final*

Proposal Title

Evaluation of the breakdown curve of OTC in the plant for control of HLB in the field (24-004)

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
02/20/2025	Citrus Research and Development Foundation	CLas Bacteria

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

1. Please state project objectives and what work was done this quarter to address them:

The objectives of this project are 1) to determine the efficacy of OTC that has been exposed to field conditions on HLB control in the field and bacterial inhibition in the lab, 2) to assess the breakdown of OTC solution when exposed to varying durations of field conditions and to determine how and when OTC breaks down within the plant tissue after injection, 3) to develop a set of guidelines for growers to increase the effectiveness of OTC injections. The overall goal of this project is to maximize the efficacy of highly labor-intensive OTC injections.

This quarter (Q1), we collected leaf tissue samples from trees that were injected with OTC solutions (exposed to 0, 1.5, 3, 6, 24, and 48 hours of field conditions) during July 2024. These trees were also rated for HLB severity, canopy color, and foliar density on a monthly basis. The leaf samples collected were used for two separate analyses - qPCR to assess CLas titers and europium extraction to quantify OTC.

The leaf samples from time zero (July 2024) and 3 months post-injection (October 2024) were processed (chopped, weighed, lyophilized, DNA extracted, and qPCR performed), and Ct values were obtained (objective 1).

The leaf samples from time zero and 24, 48, 72, and 168 hours post-injection have all been chopped, weighed, and lyophilized in duplicate. A selected subset (approximately half) of those samples have gone through the OTC extraction process. Those extraction solutions have begun to be cleaned up via multi-step filtration and subsequently measured using a plate reader (objective 2).

Initial findings from this project were published in the "Tip of the Week" in Citrus Industry magazine, which is widely available to growers (objective 3; look it up at https://citrusindustry.net/2025/01/21/oxytetracycline-efficacy-impacted-exposure-field-conditions/#:~:text=Based%20on%20numerous%20research%20studies,Figure%201).

In brief, four months post-injection, the freshly prepared OTC was the only treatment with a significant reduction in CLas titer compared to the water-injected control. This means that even as little as six hours of exposure did not provide sufficient HLB control in the grove.

2. Please state what work is anticipated for next quarter:

Objective 3) Data will be analyzed as it is processed to be ready for use in presentations and publications. Initial findings will be presented at the APS Southern Division Meeting, Florida Citrus Growers' Institute, and SWFREC's Citrus Field Day.

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Sponsor Project Number 24-004

Project Duration (years) *Year of Project* 2025

% Completion of Objectives (FDACS requirement) 4,000.00%

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.