# CITRUS ADVANCED TECHNOLOGY PROGRAM

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

**▶PROJECT QUARTER END** May 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) |
|---|---|---|
| 05/30/2025 | 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 (Q2), the leaf samples 4, 7, and 8 months post-injection (November 2024 and January & February 2025) were collected and are in the processing pipeline (chopping, weighing, lyophilization, DNA extraction, and qPCR of all samples) with Ct values already obtained from 4 months post-injection (objective 1). Additionally, monthly visual disease ratings (HLB severity, canopy color, and canopy density) data have been collected, organized, and are being analyzed. Harvest data (yield and fruit quality) have been collected along with final tree measurements (objective 1). Even in the trial with additional time points of 1.5 and 3 hours of exposure, only trees injected with fresh OTC yielded fruit with an improved Brix/Acid ratio compared to the water-treated control. Consistent differences in yield between treatments were not observed, but this may be due to extremely low yields (less than 8 lbs. per tree) across all treatments.

Bacterial inhibition assays in the lab, zone of inhibition plate assays, and kinetic growth assays in broth were conducted using OTC solutions with and without the addition of ascorbic acid that had been exposed to 0, 1.5, 3, 6, 24, and 48 hours of 30°C (objective 1). To determine if ascorbic acid could help with the uptake and distribution of OTC, replicated bench-top (detached leaf assay) and greenhouse experiments were conducted. Results from the bacterial inhibition assays were inconsistent but show that ascorbic acid does not inhibit bacteria on its own and also does not interfere with OTC's ability to inhibit bacteria. The detached leaf assay and greenhouse experiments showed that ascorbic acid caused little to no phytotoxicity and may help to increase uptake of the solution. While further validation is needed, these results were enough to move forward with putting ascorbic acid treatments in the upcoming field trial.

OTC extracts from all selected leaf samples were cleaned up via a multi-step filtration process, and OTC levels were measured using the Europium method. A subset of these clean extracts was taken to the Citrus Research and Education Center, where, along with solution samples from each time point, they were run through an HPLC machine to determine the quantity of intact OTC in each sample (objective 2). Solution samples from each time point all had clear peaks where OTC was present and showed a trend with decreased OTC in samples that had been exposed to high temperatures and UV radiation for longer periods of time. Leaf samples had more background noise but still had detectable OTC peaks. A conversion factor or formula to scale the Europium



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% Completion of Objectives (FDACS requirement) 50.00%




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