CITRUS ADVANCED TECHNOLOGY PROGRAM

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

PROJECT QUARTER END November 2025 ○ *Quarterly Report* ● *Final*

Proposal Title

A High-Throughput Screen for Natural Antibacterial Agents Against CLas

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
12/9/2025	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:

Objective 1: Screening of Natural Edible Resources for their Antibacterial Effects Against CLas.

Objective 2: Exploration of Synergistic Effects of Natural Antibacterial Agents in Combination with Oxytetracycline (OTC).

Work Accomplished This Quarter (Final Quarter):

In this final quarter, we completed the experimental work proposed in the previous report, with a focus on dose-effect characterization and synergy testing for the lead compounds identified earlier.

For Objective 1, we conducted concentration-effect (dose–response) assays for OTC and the five most effective natural compounds. All agents were tested at concentrations 10-fold lower than those used in the original screening assays. To our surprise, the five leading compounds maintained, and in several cases exceeded, the antibacterial inhibitory effect of OTC at these reduced concentrations when evaluated against BT-1 cells. These findings indicate that the lead natural products may have equal or greater potency than OTC on a per-concentration basis under our in vitro conditions.

For Objective 2, we performed synergistic effect studies by combining each of the lead natural compounds with OTC at 50% of its standard concentration. As anticipated based on their strong individual activity, the combinations produced enhanced inhibition of BT-1 growth compared with either the reduced-dose OTC or the natural compounds alone, supporting the presence of additive or synergistic interactions.

With the completion of these dose-response and combination assays, all planned laboratory work for this project has been completed. We now have a full dataset covering initial screening, identification of 4-5 lead compounds, dose-effect characterization, and preliminary synergy with OTC. Notably, the five top-performing compounds that outperformed OTC at lower concentrations have been prioritized for follow-up field validation through the Grove-First program.

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

All experimental objectives have been met, and no additional lab work is planned for the next quarter. The upcoming period will focus on:

- Compiling, organizing, and analyzing all experimental data generated over the course of the project.
- Preparing and submitting the final technical report to CRDF.
- Coordinating with the Grove-First program to initiate or plan field-level testing of the five most promising compounds, including discussions of formulation, application strategies, and integration with existing OTC management practices.

3. Please state budget status (underspend or overspend, and why):

By the end of this final quarter, all budgeted funds have been fully expended as planned. The 6-month no-cost extension allowed us to complete all proposed experimental work, including the expanded screening, dose-response assays, and synergy studies. Spending is on target with the approved budget, with no significant underspend or overspend anticipated at project close.

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Sponsor Project Number 23-027

Project Duration (years) 1.5 *Year of Project* 1.5

% Completion of Objectives (FDACS requirement) 100%

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.