CITRUS ADVANCED TECHNOLOGY PROGRAM

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.

▶PROJECT QUARTER END November 2025

○ *Quarterly Report* ● *Final*

Proposal Title

A High-Throughput Screen for Natural Antibacterial Agents Against CLas

Today's Date: 12/9/2025

Sponsoring Organization (drop-down): Citrus Research and Development Foundation

Category (drop down): 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 during the entire project to address them:
Objective 1: Screening of natural, edible resources for their antibacterial effects against Candidatus Liberibacter asiaticus (CLas), using Liberibacter crescens BT-1 as a surrogate. Objective 2: Exploration of synergistic effects of natural antibacterial agents in combination with oxytetracycline (OTC). Work Accomplished (Summary of Entire Project): Across the project period (including the no-cost extension), we established and refined a BT-1–based in vitro assay to evaluate natural products for anti-CLas activity and to compare them with OTC. Early quarters focused on: 1. Developing and optimizing the BT-1 assay, including culture conditions, incubation time, and readout (growth inhibition) to ensure reproducible screening. 2. Establishing OTC at 0.1 µg/mL as a baseline reference for antibacterial activity. 3. Building a library of natural materials (extracts and purified compounds) from edible resources. These materials were prepared using solvents of varying polarity (e.g., ethyl acetate, methanol, water) and purified where needed using chromatographic methods (e.g., size-exclusion and polarity-based column separations). Over subsequent quarters, we: a. Extracted, purified, and assembled a panel of 77 distinct natural product extracts/compounds, and then expanded this to approximately 85 total materials, in line with the original project target. b. Screened the compound library against BT-1, initially at higher test concentrations to identify candidates with measurable growth inhibition relative to the OTC baseline and solvent controls. c. From this screening, we narrowed the list to a subset of active compounds, and then further refined this to five lead compounds that consistently inhibited BT-1 growth across repeated experiments. d. As BT-1 grows slowly and is prone to contamination, progress required extended incubation periods and careful culture management, which led to a 6-month no-cost extension to complete all planned assays. In the final two quarters, we focused on completing the most critical parts of both objectives: We performed dose-response experiments for OTC and the five lead natural compounds, testing them at 10-fold lower concentrations than those used in the initial screens. Surprisingly, all five lead compounds maintained strong inhibition of BT-1 growth at these lower concentrations and, in several cases, showed better inhibition than OTC at its reduced dose. These results indicate that the best natural compounds are at least as potent as OTC on a per-concentration basis under our in vitro conditions. We then evaluated combinations of each lead compound with OTC at half of its standard concentration(i.e., 50% of the established baseline dose). As anticipated based on their strong individual activity, the combinations produced greater inhibition of BT-1 growth than either the half-dose OTC or the natural compounds alone, supporting additive or synergistic interactions. These findings support the feasibility of using these natural compounds to reduce the required OTC dose while maintaining or improving antibacterial efficacy. By the end of the final quarter, all planned laboratory tasks were completed: 1. The full ~85-compound library was screened. 2. A set of five top candidate compounds was defined and validated across repeated assays and dose–response testing. 3. Synergistic effects with reduced-dose OTC were demonstrated.

2. Please state what work is anticipated after project completion: All experimental objectives for this CRDF project have been achieved, and no additional CRDF-funded laboratory work is planned. The next steps beyond this project are Field validation through Grove-First:The five most effective natural compounds, which showed equal or better inhibition than OTC even at lower concentrations, will be advanced to field testing via the Grove-First program.

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Organization: UF/CREC

Sponsor Project Number: 23-027

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

% Completion of Objectives (FDACS requirement) 100%