# CITRUS ADVANCED TECHNOLOGY PROGRAM

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

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

Proposal Title

Optimal combination of Bt toxins and gene silencing RNAs for management of citrus root weevil

| Today's Date | Sponsoring Organization (drop-down) | Category (drop down) |
|---|---|---|
| 05/07/2025 | Citrus Research and Development Foundation | ACP Vector |


**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. Bacterial Pesticidal Proteins**: Five additional bacterial pesticidal proteins (BPP) have been expressed under optimized conditions and purified for testing against DRW. In preliminary bioassays, the toxicity of 16 BPP was tested by droplet feeding of larvae (3-4 month old) with 20 ppm of BPP with or without an 8- or 24-hour period of starvation to facilitate ingestion of the BPP. In this initial screen, six BPP resulted in DRW mortality within 7 to 10 days. As it takes 6 to 7 months for neonate larvae to reach the adult stage, multiple asynchronous colonies of DRW have been established to ensure ready availabilty of larvae for these bioassays.

**Objective 2**. **Gene Silencing RNAs:** As genomic resources for DRW were minimal, a comprehensive transcriptome database for all life stages was generated to facilitate identification of target genes for silencing for this project. Thirteen target genes were selected and dsRNAs produced. In preliminary bioassays, larvae (2-3 months old) were droplet fed on 10 to 200 ng of test or negative control (GFP) dsRNA. Changes in growth, development, and mortality relative to the GPF dsRNA treatment and untreated control were recorded. Significant impacts on growth and development were noted for several dsRNA treatments. Transcription of each of the 13 target genes during each DRW life stage was quantified. Genes that were primarily transcribed in a single life stage were eliminated from further consideration to avoid life stage-dependance of gene silencing impacts on DRW.

**Objective 4: Assess four Bt transgenic citrus lines for DRW resistance**: A plant transformation construct was produced for expression of two separate bacterial pesticidal proteins in the same plant under control of the 35S promoter. Of 34 Swingle lines generated, 21 were selected for molecular analysis based on vigor and reporter gene expression. Transcription of each BPP gene was confirmed in the majority of these lines, and cuttings are now being propagated for further analyses and testing for resistance to DRW.

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

Bioassays to screen for the impacts of BPP (objective 1) and dsRNA (objective 2) on DRW larvae will be continued using the protocols optimized during the past quarter.

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

Budget on track.



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Sponsor Project Number 23-040

Project Duration (years) 3 *Year of Project* 1

% Completion of Objectives (FDACS requirement) 28.00%




CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.