Assessing therapeutic effects of CitruSaver on the productivity and health of HLB-affected citrus trees

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Progress Report: Fall 2024 – Spring 2025

Executive Summary

This report outlines the progress made in the CitruSaver Project titled "*Assessing therapeutic effects of CitruSaver on the productivity and health of HLB-affected citrus trees*" across multiple sites in Florida and Texas from Fall 2024 to Spring 2025. This collaborative project is focused on assessing the therapeutic effects of CitruSaver and beneficial microbes on the productivity and health of HLB-affected citrus trees and improving citrus-tree performance. The project is currently being conducted in commercial citrus groves across Florida and Texas, focusing on three important citrus varieties: two sweet orange varieties (Valencia and Hamlin), and also grapefruit. Both commercial Valencia field-trial groves in Florida received 2 years of treatments with OTC (oxytetracycline) trunk injections. The Texas Valencia commercial grove, as well as the Texas grapefruit commercial grove, were not injected with OTC. This report covers activities carried out at each site, data collected, current findings, and challenges encountered. It also provides a detailed plan of the work to be completed in the next project phase.

Project Initiation and Field Setup

The project officially began in Fall 2024 with field surveys and the selection of study trees. During this initial stage, emphasis was placed on ensuring experimental consistency across the various field sites. Uniform citrus trees, with similar age, canopy size, and visible health, were selected and permanently flagged (Figure 1). Mapping of all selected trees was carried out to facilitate monitoring and data collection over time. This setup phase also involved building relationships with the participating growers in both states. These interactions were instrumental in understanding site histories, current management practices, and logistical considerations. Some variability in management between growers, particularly in irrigation, was noted early and continues to present both a challenge and a learning opportunity.

Figure 1. Tree selection and flagging in Florida and Texas.

Baseline Sampling

To establish a reference point (Time 0) for future comparisons, preliminary data were collected prior to any treatment applications. Soil samples were collected at multiple depths (0-15 cm and 15-30 cm) to analyze initial nutrient levels, soil pH, soil organic matter, and baseline microbial populations (Figure 2). Simultaneously, tissue samples from leaves were gathered to evaluate the internal nutrient status of the trees before the treatment effect and the *Candidatus* Liberibacter titer (*C*Las) titer of the leaves. This pre-treatment dataset provides the baseline needed to assess the effectiveness of treatments over the project duration. These baseline samples were completed for all experimental blocks in both Florida and Texas, offering a comprehensive picture of initial soil and plant health across sites.

Citrus Varieties and Fruit Drop Assessment

The project encompasses three key varieties: Valencia (in both Florida and Texas), Hamlin (Florida), and Grapefruit (Texas). Each variety presents unique physiological traits and management challenges, making this a valuable comparative study.

Figure 2. Soil and leaf tissue sampling process.

Of note, the Valencia blocks in Florida provided the first opportunity to assess fruit retention before harvest. A subset of fruit drop data was collected prior to harvesting, focused solely on the Valencia variety (Figures 15-16). Although this data set was partial, it allowed us to begin quantifying the extent of fruit drop and exploring how it may correlate with baseline conditions or early- stage treatments. Several trees showed unusually high fruit drop, and these cases are being closely monitored moving forward.

Treatment Applications and Site Management

Following the collection of baseline data, the first round of soil amendment and microbial treatments was done, beginning with the Valencia blocks in Florida. Citrusaver 1 and Citrusaver 2 were applied over the course of nine weeks. The application schedule included three root zone applications and one foliar application, scheduled at two-week intervals. The Fort Meade irrigation Valencia block is 10 acres (~1,300 trees) and has received continuous CitruSaver treatments through micro jet irrigation for the past four years, whereas all other treatments were applied for the first time this year to previously untreated ("virgin") trees. The Wauchula irrigation Valencia block is 17 acres (~2,175 trees) and began its first CitruSaver treatments this

year. All the trees in the irrigation blocks received treatments via micro jet irrigation.

Figure 3. Fruit drop measurement.

The formulations contain a combination of biostimulants designed to improve nutrient cycling, enhance soil structure, and stimulate plant growth. In addition to the biostimulants, two strains of beneficial microbes, *Azotobacter chroococcum* and *Pseudomonas protegens*, were applied to the soil and root zone before harvesting. These organisms are known to promote plant growth, enhance nitrogen fixation, improve phosphorus cycling, and suppress harmful pathogens. The Texas blocks are currently undergoing the same treatment as of late Spring 2025. The same treatment protocol has now been initiated in the Hamlin blocks in Florida sites.

Figure 4. Root zone and foliar applications.

Laboratory Analyses and Data Collected
Several types of data have been collected throughout the project to track the effects of treatments and natural environmental variability.
Fruit Yield and Juice Quality: The first full harvest was conducted in the Valencia blocks. Yield was measured per plot, and fruit samples were processed to assess juice quality

parameters, including total soluble solids (°Brix), titratable acidity, and the Brix-to-acid ratio, key indicators of market quality and consumer preference.

Canopy Measurements: Canopy width and density measurements were taken manually and with digital tools to evaluate structural growth responses to treatment. These data helped quantify above-ground vigor and support estimations of photosynthetic potential. These measurements were taken from both the Texas and Florida groves. In Texas, preliminary canopy measurements were taken on young Valencia and mature grapefruit (Figures 7 and 8). In Florida, these measurements were from the Valencia field trials in Fort Meade and Wauchula (Figures 5 and 6).
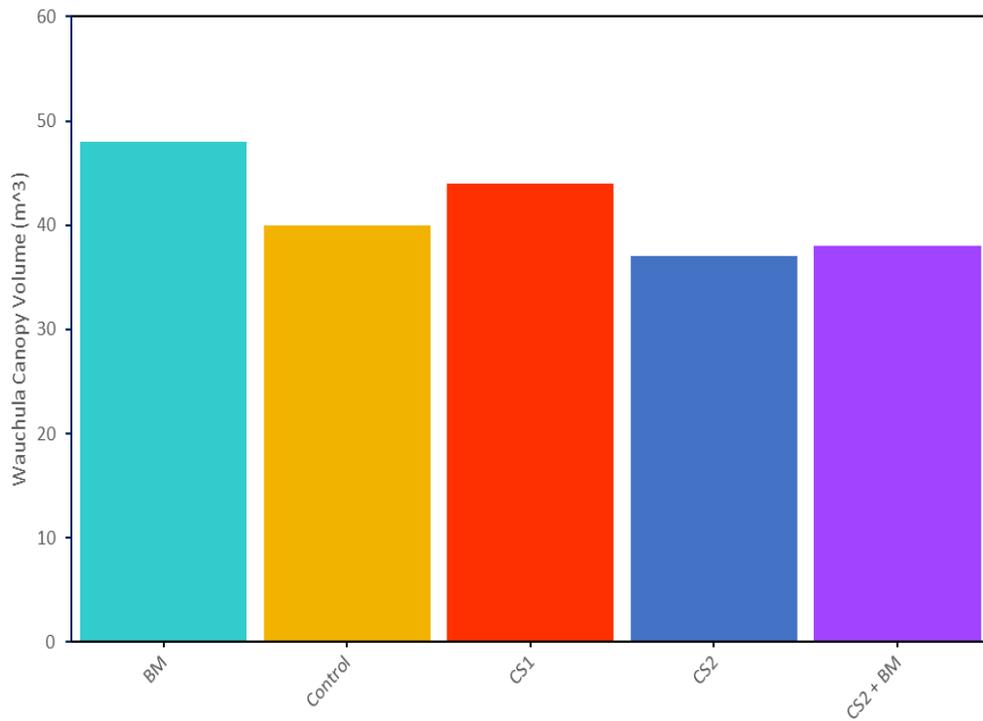

Figure 5: Wauchula Canopy Volume Comparison. Measurements taken on 6/6/2025 (approximately 4 months after the initial date of CitruSaver treatment applications).
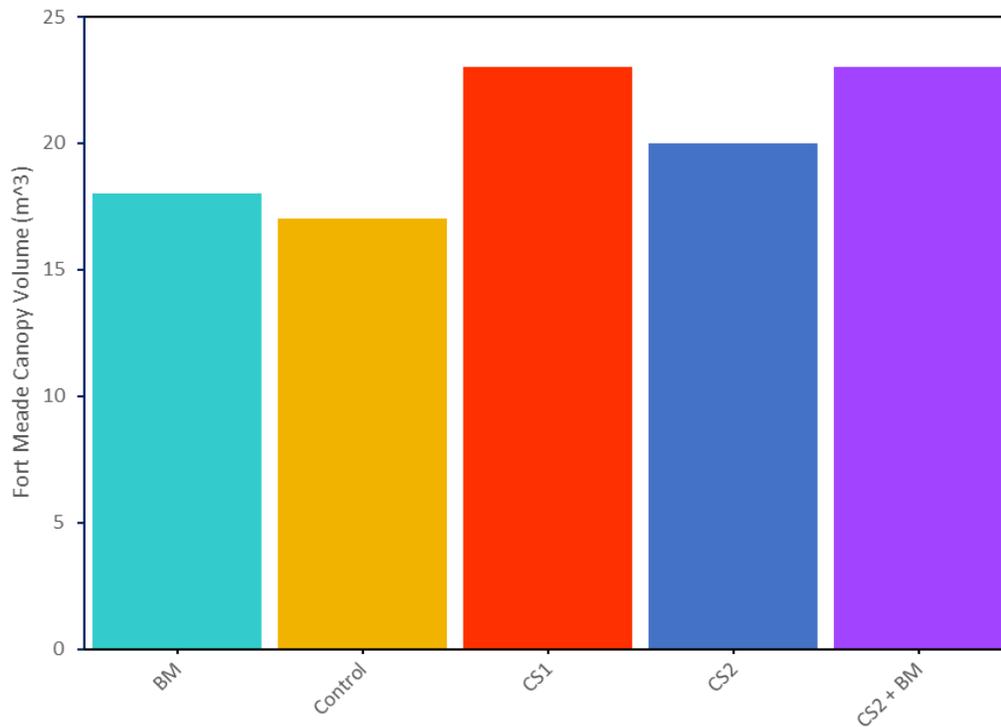
Figure 6: Fort Meade Canopy Volume Comparison. Measurements taken on 6/20/2025 (approximately 5 months after the initial date of CitruSaver treatment applications).

The comparison of canopy volume results between CS1 and Control across both Wauchula and Fort Meade locations reveals distinct differences. In Wauchula, CS1 exhibits a canopy volume of approximately 44 m³, higher (+10%) than the Control's 40 m³. Similarly, in Fort Meade, CS1 shows a canopy volume of about 23 m³, notably exceeding the Control's 17 m³ (+35%). These findings suggest that the CS1 condition consistently enhances canopy volume compared to the Control condition in both locations.
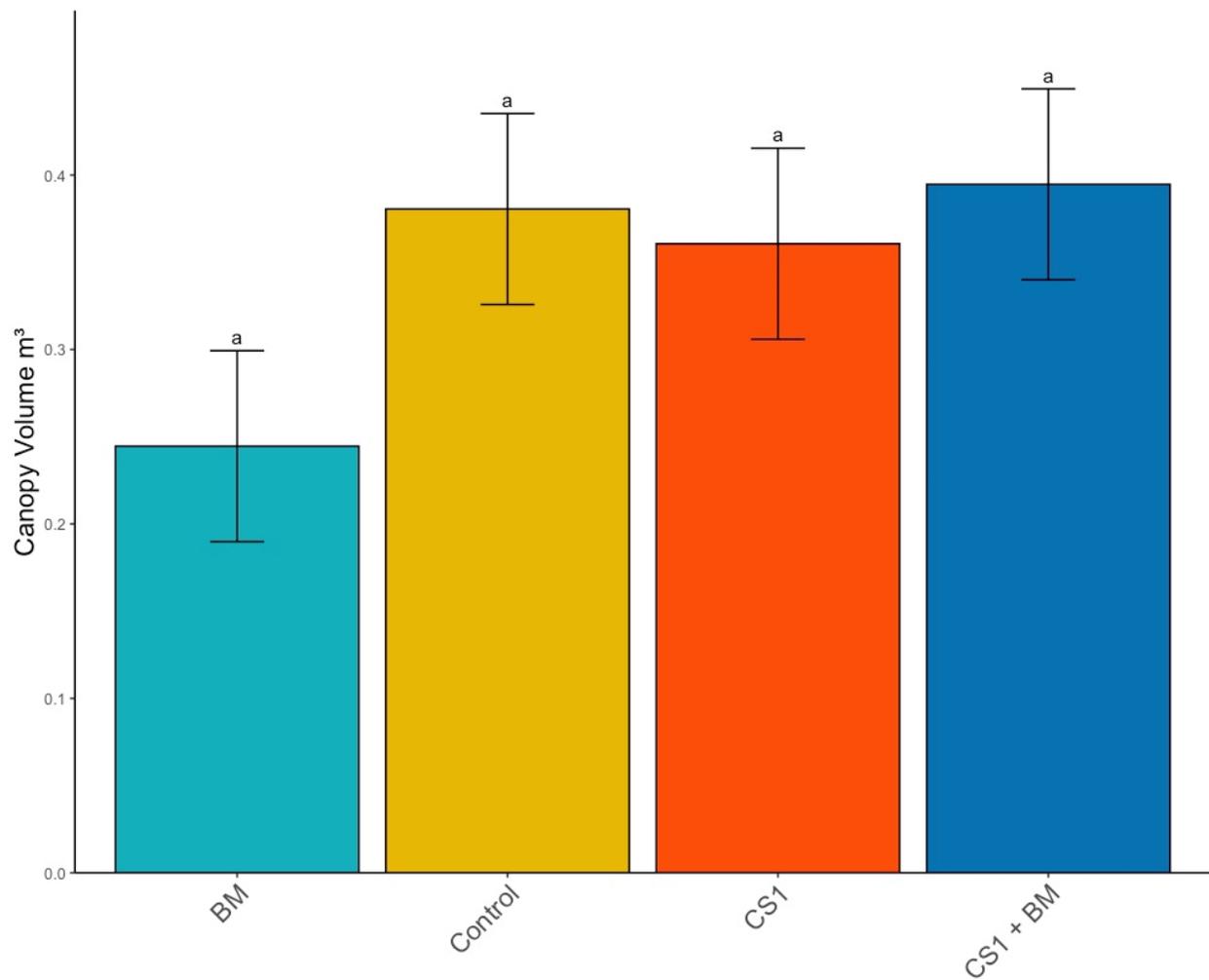
Figure 7. Trends in Texan young Valencia citrus trees' canopy volume. Mean values ± standard error with the same letters are not significantly different. Mean separation was made at p≤0.05 using Tukey's honest significant difference method. A second tree canopy volume measurement will be conducted during the week of 9/22/2025.
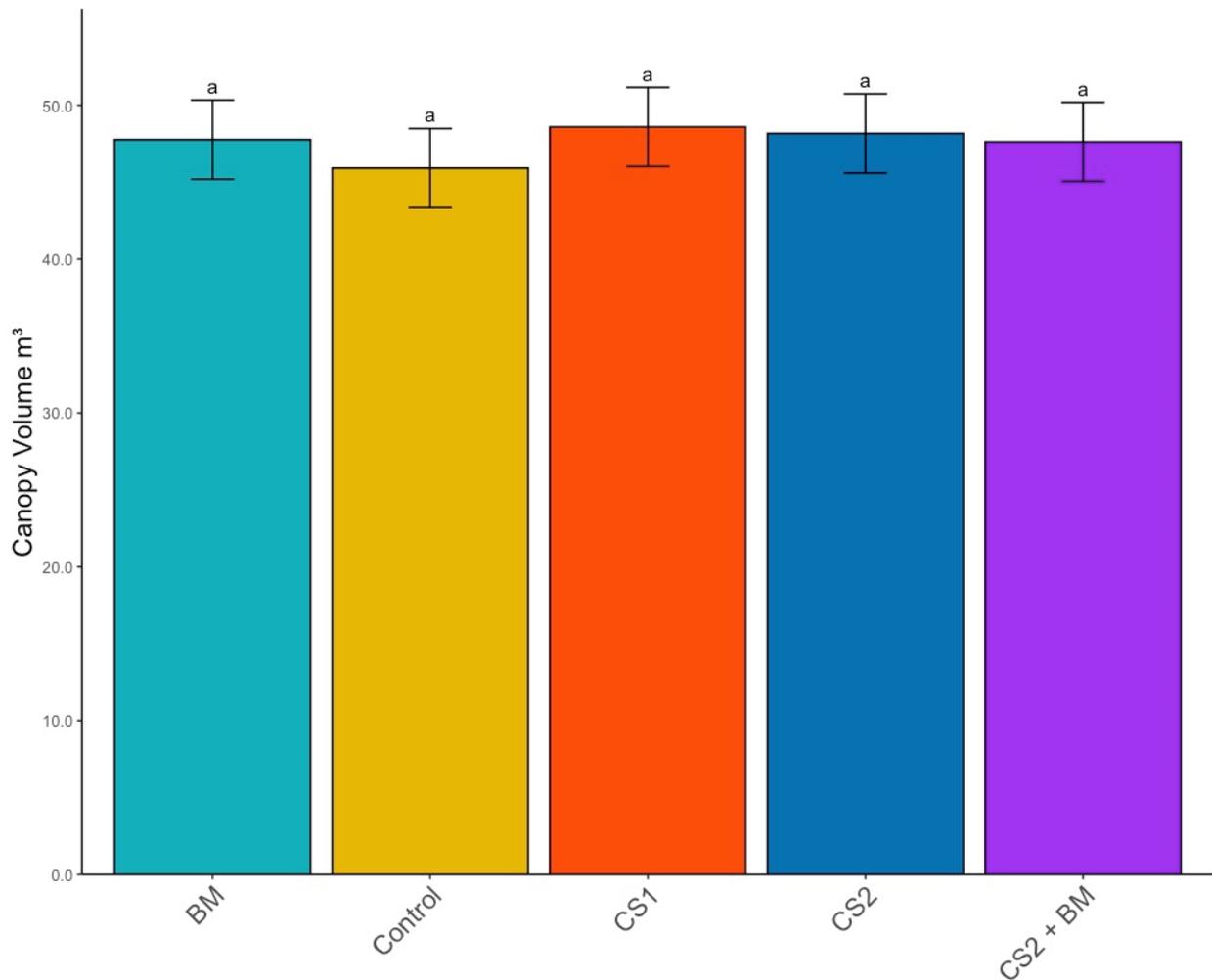.

Figure 8. Trends in Texan Grapefruit citrus trees' canopy volume. Mean values ± standard error with the same letters are not significantly different. Mean separation was made at p ≤0.05 using Tukey's honest significant difference method. A second tree canopy volume measurement will be conducted during the week of 9/22/2025.

Fruit Drop and Root Health: Preliminary fruit drop counts (Figure 15) were conducted in select plots, and root sampling was performed in Spring 2024. These samples are currently being stored and will be processed after the greenhouse experiment is fully established.
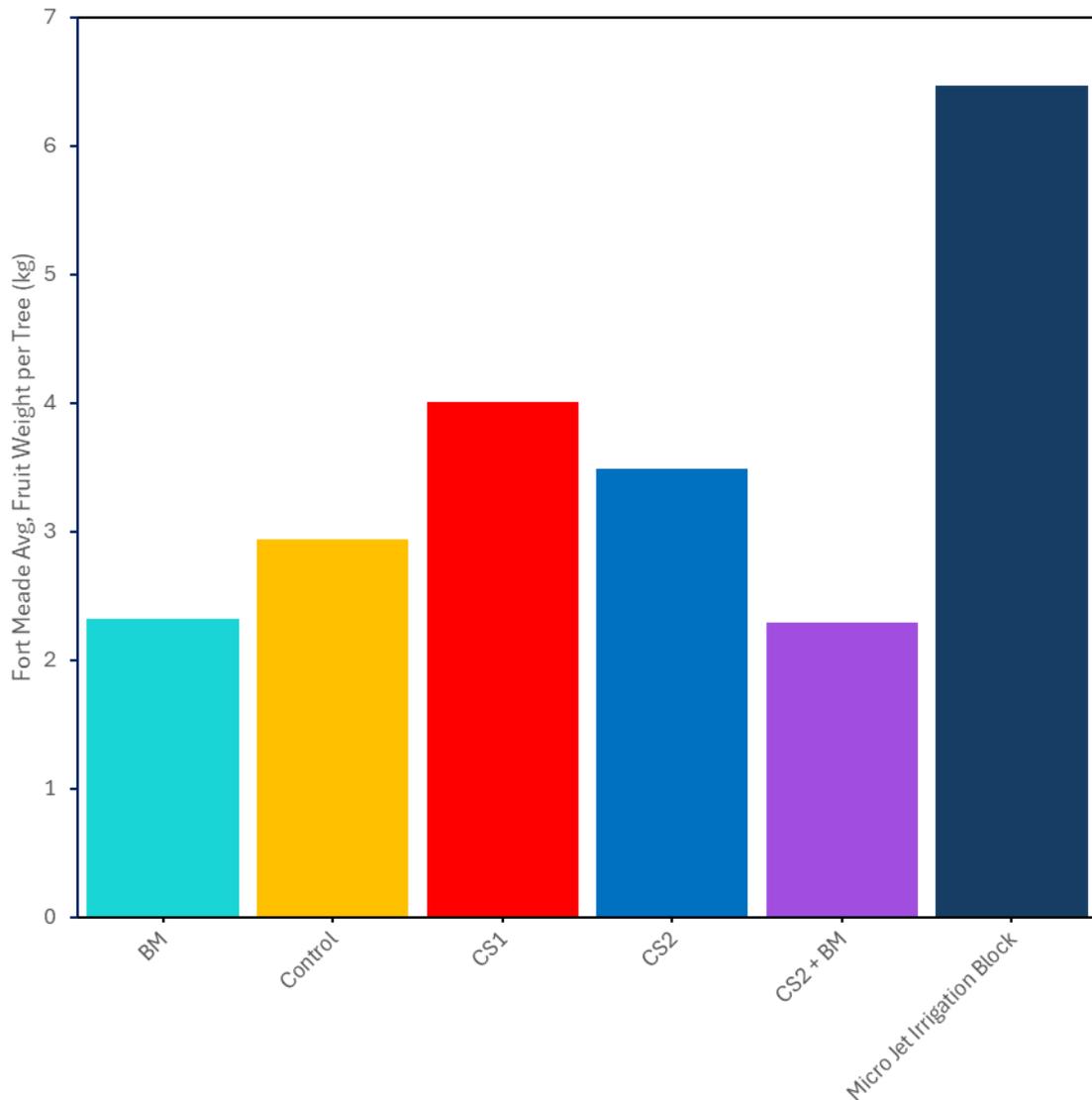
Figure 9. Average weight of picked fruit per tree (kg) for different treatments at the Fort Meade grove. Harvest date was 4/29/2025 (approximately 3 months after the initial date of Citrusaver treatment applications). Decreased harvest yields were a result of the late fruit picking. This late harvest was caused by the juice processing plant's delayed schedule. Treatments include BM, Control, CS1, CS2, CS2 + BM, and irrigation through micro jet. The 10 acre irrigated through micro jet already four years running, produced the highest average weight of picked fruit per tree, followed by CS1.

- **The Fort Meade micro jet-irrigated Valencia block is 10 acres (~1,300 trees) and has received continuous CitruSaver treatments for the past four years**

Fort Meade	Citrusaver 1	Citrusaver 2	Citrusaver 2 + Microbes	Microbes	Micro Jet Irrigation Block	Control
Total Harvest weight from 12 trees per treatment (in pounds)	106.04	92.36	60.68	61.56	171.072	77.84
Difference by Percentage CitruSaver treatment vs untreated control	36%	19%	-22%	-21%	120%	
Fruit Yield per tree in LBS	8.84	7.70	5.06	5.13	14.256	6.49
Pounds Solids per 90 Lb box	5.44	5.19	5.19	5.34	4.93	5.20
Pounds Solids per tree	0.53	0.44	0.29	0.30	0.78	0.37
Difference by Percentage: Treatments vs untreated control (lb solids/tree)	43%	19%	-22%	-19%	109%	

Table 10. Fort Meade Valencia yield factors. CitruSaver 1 treatment achieved a 43% improvement over control, and the irrigated trees on their fourth year had a 109% improvement vs control.

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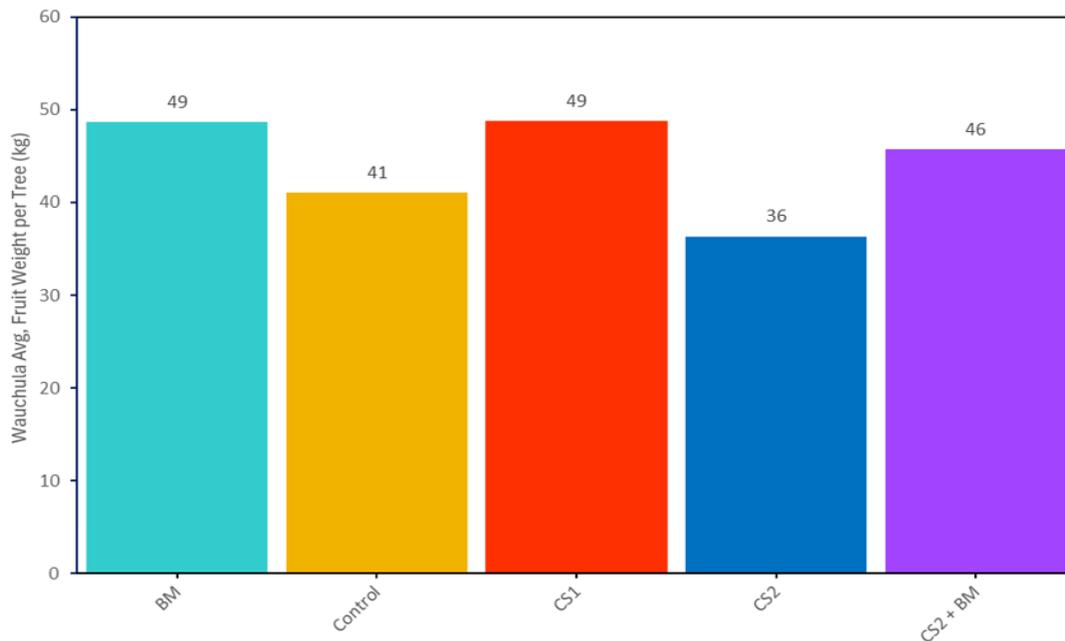

Figure 11. Average weight of picked fruit per tree (kg) for different treatments at the Wauchula grove. Harvest date was 3/26/2025 (approximately 2 months after the initial date of CitruSaver treatment applications). Treatments include BM, Control, CS1, CS2, and CS2 + BM. CS1 produced the highest average weight of picked fruits per tree, followed by BM. As a reference, the Florida average Valencia fruit yield was 38 kg/tree for groves harvested during the same harvest date.

Wauchula	Citrusaver 1	Citrusaver 2	Citrusaver 2 + Microbes	Microbes	Control
Total Harvest weight from 12 trees per treatment (in pounds)	1287.4	959.2	1207.6	1285	1084.2
Difference by Percentage: Treatments vs untreated control (total yeild)	19%	-12%	11%	19%	
Fruit Yield per tree in LBS	107.2833333	79.93333333	100.6333333	107.0833333	90.35
Pounds Solids per 90 Lb box	5.56	5.46	5.51	5.55	5.50
Pounds Solids per tree	6.63	4.85	6.16	6.60	5.52
Difference by Percentage: Treatments vs untreated control (lb solids/tree)	20%	-12%	11%	20%	

Table 12. Wauchula Valencia fruit yield figures per tree in lb. solids. CitruSaver 1 treatment achieved a 20% improvement over control, and the Beneficial Microbes achieved a similar result.

Fruit Yield: Across both trial locations, the CS1 treatment consistently produced the highest average weight of picked fruit per tree compared with the untreated control and other treatments. At Fort Meade, trees treated with CS1 averaged 4.02 kg per tree versus 2.95 kg for the control—a yield increase of 36%. The hectares treated four years through micro jet irrigation block at Fort Meade yielded 6.48 kg/tree, an astounding 120% increase over the control. At Wauchula, CS1 led with 48.67 kg of picked fruit per tree compared with 41.07 kg for the control, an increase of about 7.5 kg (19%) per tree. Other treatments (BM, CS2, and CS2 + BM) showed mixed results—some exceeding the control at one site but not the other—while CS1 was the only treatment to outperform the control consistently at both sites. This indicates that CS1 delivered the most pronounced yield benefit across the two locations.

Figure 13. Fruit harvest in Florida.

qPCR Analysis: All tissue samples collected after treatment application were analyzed using quantitative polymerase chain reaction (qPCR) to determine the *Candidatus* Liberibacter asiaticus (*C*Las) titer, the causal agent of Huanglongbing (HLB) or citrus greening disease. The qPCR protocol allowed for accurate quantification of *C*Las DNA in leaf samples, providing an estimate of bacterial load or "titer" in each tree. This serves not only as a disease severity indicator but also as a metric to evaluate the impact of biostimulants and microbial treatments on disease suppression or progression. Higher cycle threshold (Ct) values indicate lower bacterial load, while lower Ct values mean more advanced infection. (Figure 14).
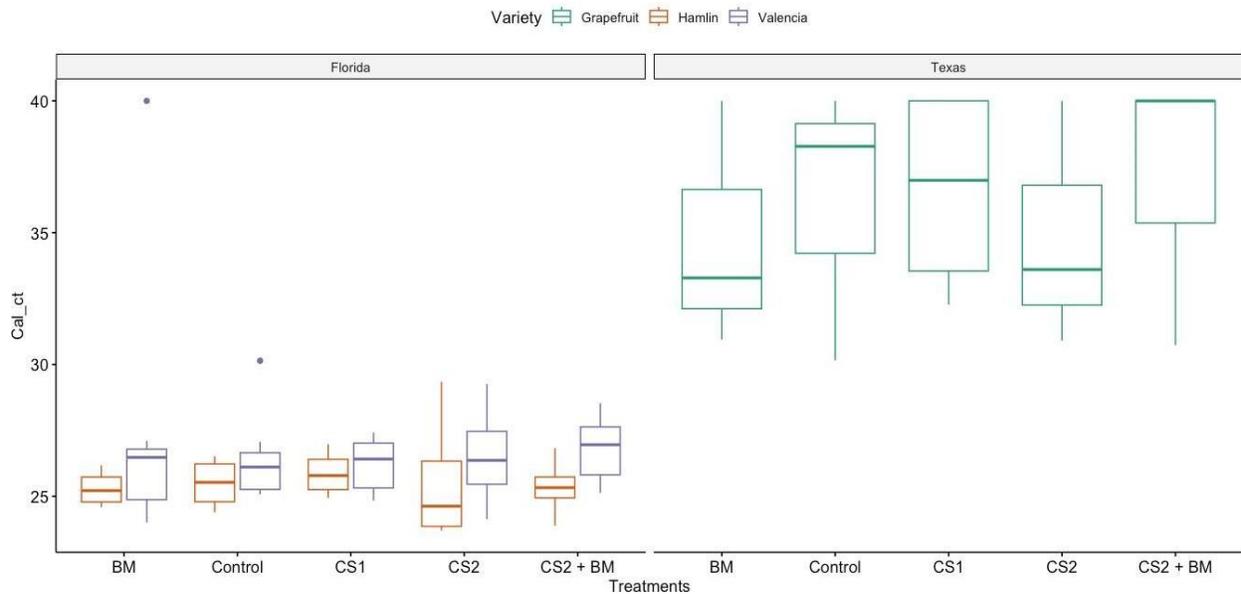

Figure 14. *C*Las titer levels in leaf tissue for all sites with the same treatments: Wauchula and Fort Meade, Florida, and Texas grapefruit, grouped into their states. Comparisons grouped for four treatments plus the control, and 3 citrus varieties. Higher Ct values indicate lower bacterial load, and lower Ct values indicate more advanced infection.
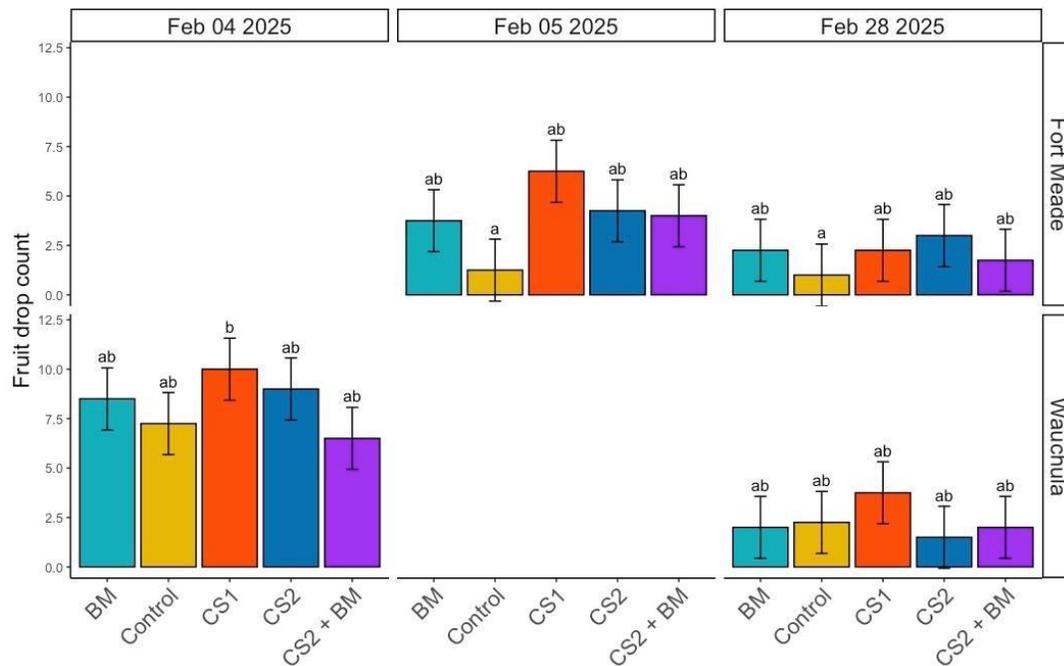
Figure 15. Fruit drop counts. Comparisons grouped into four treatments plus control, and 2 sites—Fort Meade and Wauchula. Mean values ± standard error with the same letters are not significantly different. Mean separation was done at p ≤ 0.05 using Tukey's honest significant difference method.

Challenges and Observations

Several issues have emerged during the project, which have influenced either data quality or plant performance:

1. Localized Fruit Drop: Unusual fruit drop was observed in certain Valencia trees, especially in Florida (Figure 16A). This phenomenon may be linked to soil moisture fluctuations or tree stress, and we are currently investigating this further.

2. Grower Management Variability: Inconsistent irrigation by some growers, particularly under-watering during dry periods, has affected the uniformity of treatment impacts and could compromise data interpretation. Continued grower engagement will be critical.

3. Gummosis in Grapefruit Trees: The appearance of gummosis symptoms in some grapefruit trees in Texas raises concerns about potential pathogen interference with treatment effects (Figure 16B). Monitoring and potential mitigation strategies will be required in the next phases.
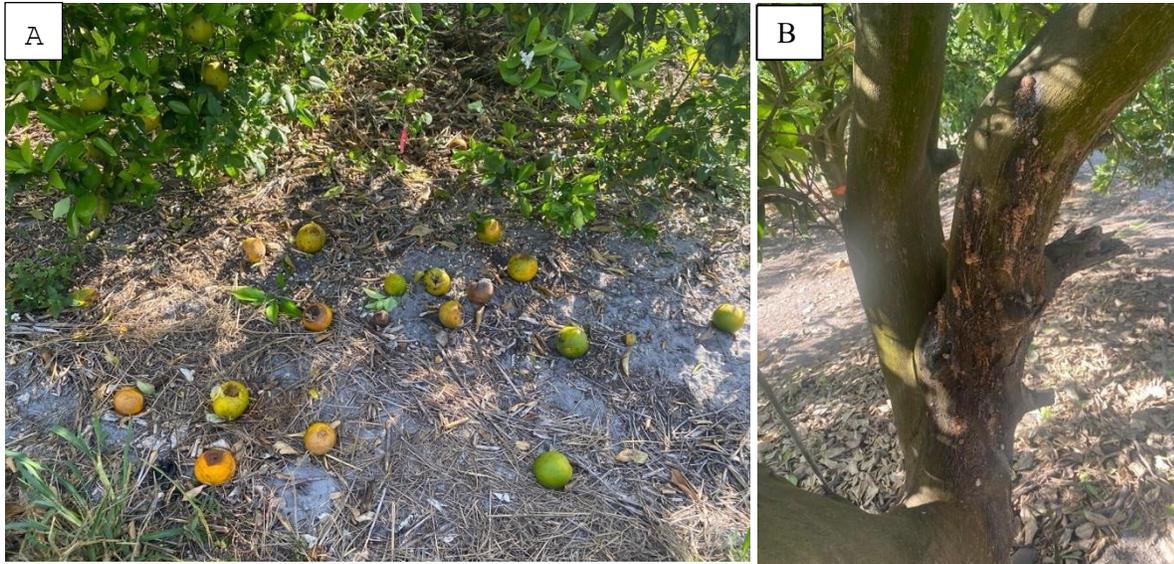
Figure 16. Observations. (A) Localized fruit drop observed with certain Valencia trees, (B) Gummosis in grapefruit trees.

Future Work Plan

Looking ahead, several key milestones are scheduled for the coming months:

Completion of Hamlin Treatment Applications: The remaining applications for the Hamlin variety in Florida will be completed in early summer.

Greenhouse Experiment Setup: A controlled greenhouse trial will be initiated by mid-August 2025. This component of the project will allow us to test treatments under standardized conditions, focusing on root development, nutrient uptake, and microbial activity.

Soil Sample Processing for Root Density: Root zone soil samples collected in Spring 2024 will be processed in the laboratory as soon as the greenhouse is operational. These analyses will provide valuable insight into CitruSaver, microbial activity, and soil structural changes.

Fall 2025 Sampling: A second round of soil and tissue sampling will be conducted at all sites to evaluate the impacts of the treatments. Additionally, fall canopy measurements will be repeated, and fruit drop squares will be installed in Hamlin plots to facilitate more structured pre-harvest assessments.

Conclusion

This project has made significant initial progress in evaluating the effects of biostimulants and beneficial microbes on citrus performance in Florida and Texas. The baseline data, treatment implementation, and early harvest results and canopy measurements have provided a solid foundation for ongoing analysis and interpretation. While challenges such as fruit drop and irrigation variability have emerged, they also provide real-world context that strengthens the value of the findings. The next phase will be critical for validating early trends and expanding our understanding of how soil and plant health can be improved in citrus systems.

As of the writing of this report, the citrus trees that have been treated with CitruSaver and beneficial microbes have demonstrated positive response to these treatments. CitruSaver 1 treated trees yielded 43% higher total pound solids per tree in the Fort Meade Valencia field trial and 20% higher total pound solids per tree in the Wauchula Valencia field trial as compared to the control trees. CitruSaver 2 treated trees yielded 19% higher total pound solids per tree in the

Fort Meade Valencia field trial and the microbes yielded 20% higher total pound solids per tree in the Wauchula Valencia field trial as compared to the control trees. Treated trees exhibited great canopy vigor and new flush growth compared to the control trees. CitruSaver 1 treated trees exhibited a 35% greater total canopy volume in the Fort Meade Valencia field trial and 10% greater total canopy volume in the Wauchula Valencia field trial as compared to the control trees.

Finally, the treated trees exhibited noticeably less branch dieback as compared to the control trees in both the Fort Meade and Wauchula Valencia field trials. This observable difference is an encouraging indicator that these treatments are having a positive effect in assisting the HLB infected trees to combat tree health decline.

Continued support and collaboration with the growers and Savory Sun VA LLC will be essential for maximizing impact and achieving the project's long- term objectives.

Appendices: Timeline of Activities

Period	Activity	Site	Notes
Fall 2024	Tree selection, grower visits	FL & TX	Baseline engagement

| Fall 2024	Soil & tissue sampling (Time 0)	FL & TX	All plots
Winter–Spring 2025	Treatment applications (Valencia)	FL	4 total applications
Spring 2025	Harvest (Valencia), Yield, Juice Quality	FL	Data collected
Spring 2025	qPCR Analysis	ALL	Completed
Spring 2025	Treatment applications start	TX	Ongoing
Current	Hamlin Applications	FL	In progress
June–Aug 2025	Greenhouse Setup	FL	Target: mid-August

Annexes
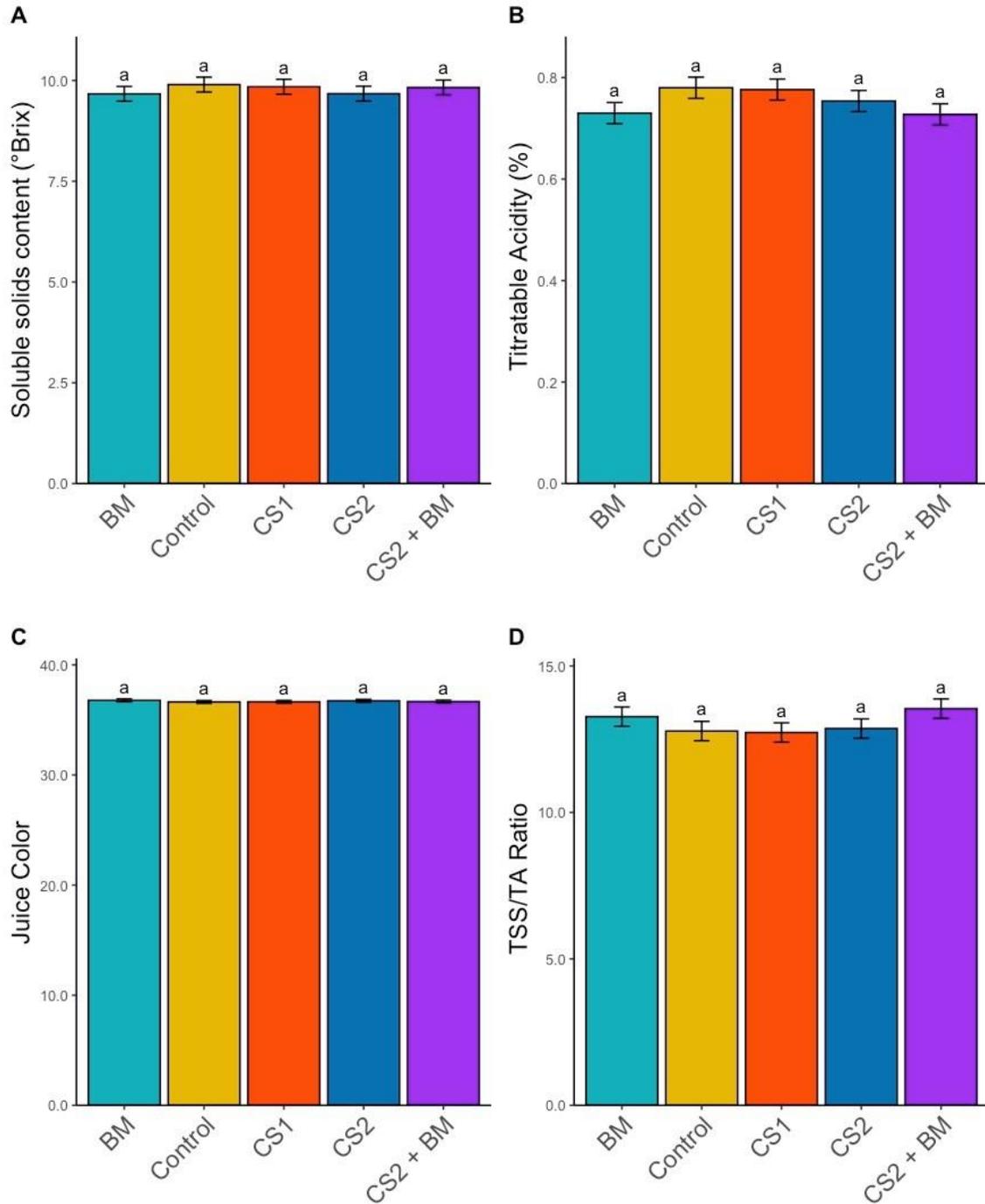
Fig 17. Juice quality analysis for Fort Meade and Wauchula Valencia sites for (A) Soluble solids content, (B) Titratable acidity, (C) Juice color, (D) Soluble solids/Titrable acidity ratio (ripening index - RI), for the four treatments plus the control. Mean values ± standard error with the same letters are not significantly different. Mean separation was done at p ≤ 0.05 using Tukey's honest significant difference method.
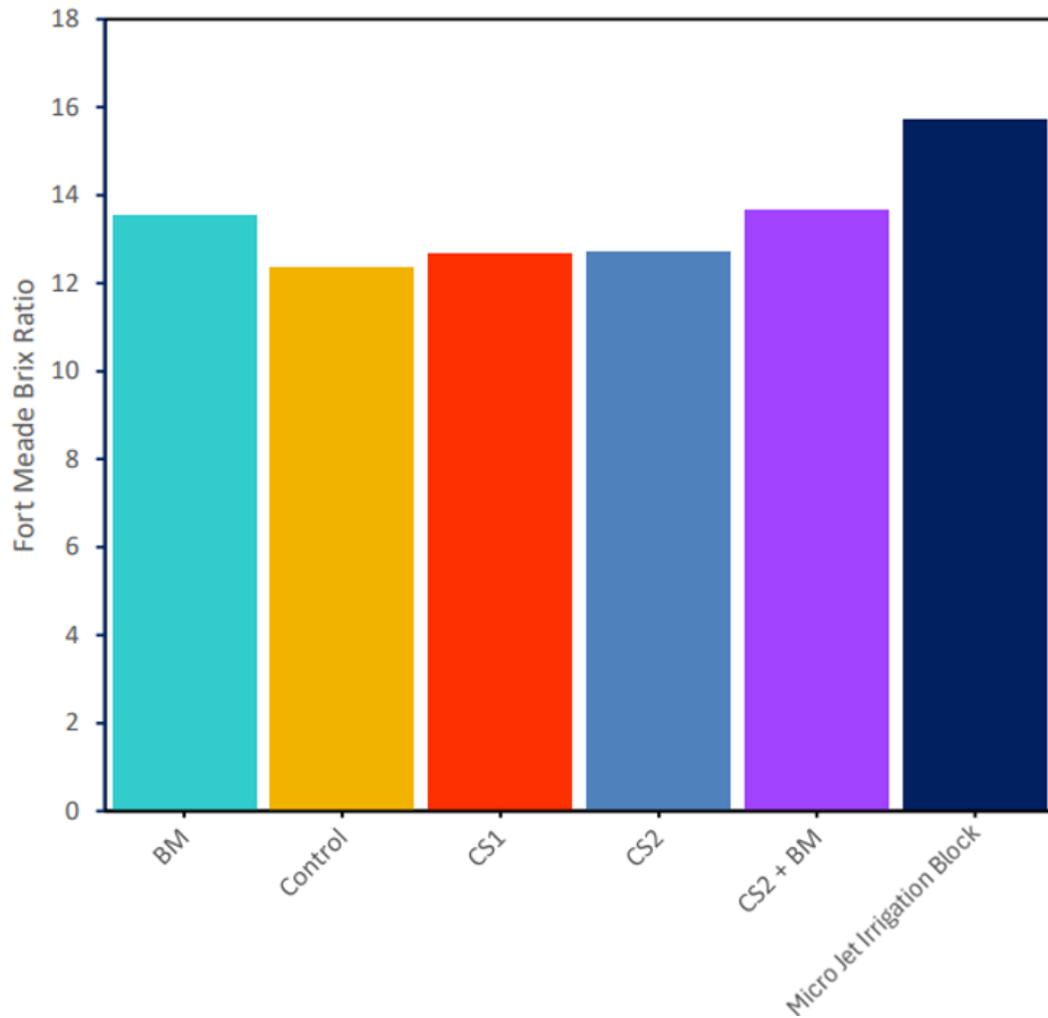
Fig 18. Average Brix ratio at Fort Meade for each treatment. Treatments include BM, Control, CS1, CS2, CS2 + BM, and micro jet irrigation. All four treatments produced higher Brix ratios compared to control. However, the treatment through micro jet irrigation, now in its fourth year, resulted in the highest Brix/acid ratio at 15.7, indicating the most favorable balance of sugars to acidity compared with the other treatments.
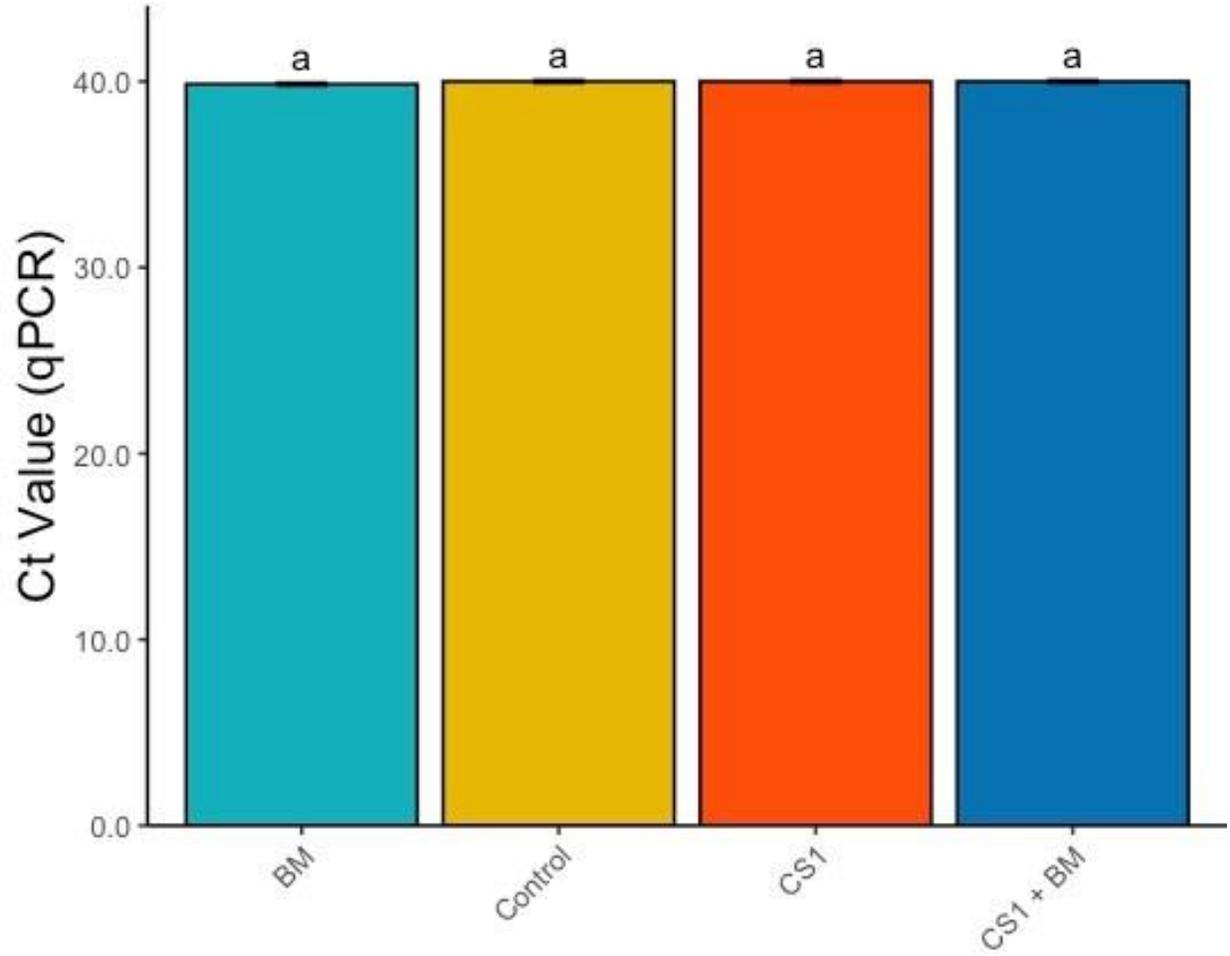
Fig 19. *C*Las titer levels in leaf tissue for Texas Valencia, later grouped into states. Comparisons for the four treatments plus the control. Higher Ct values indicate lower bacterial load, and lower Ct values indicate more advanced infection.

Fig 20. Canopy measurements in Texas.
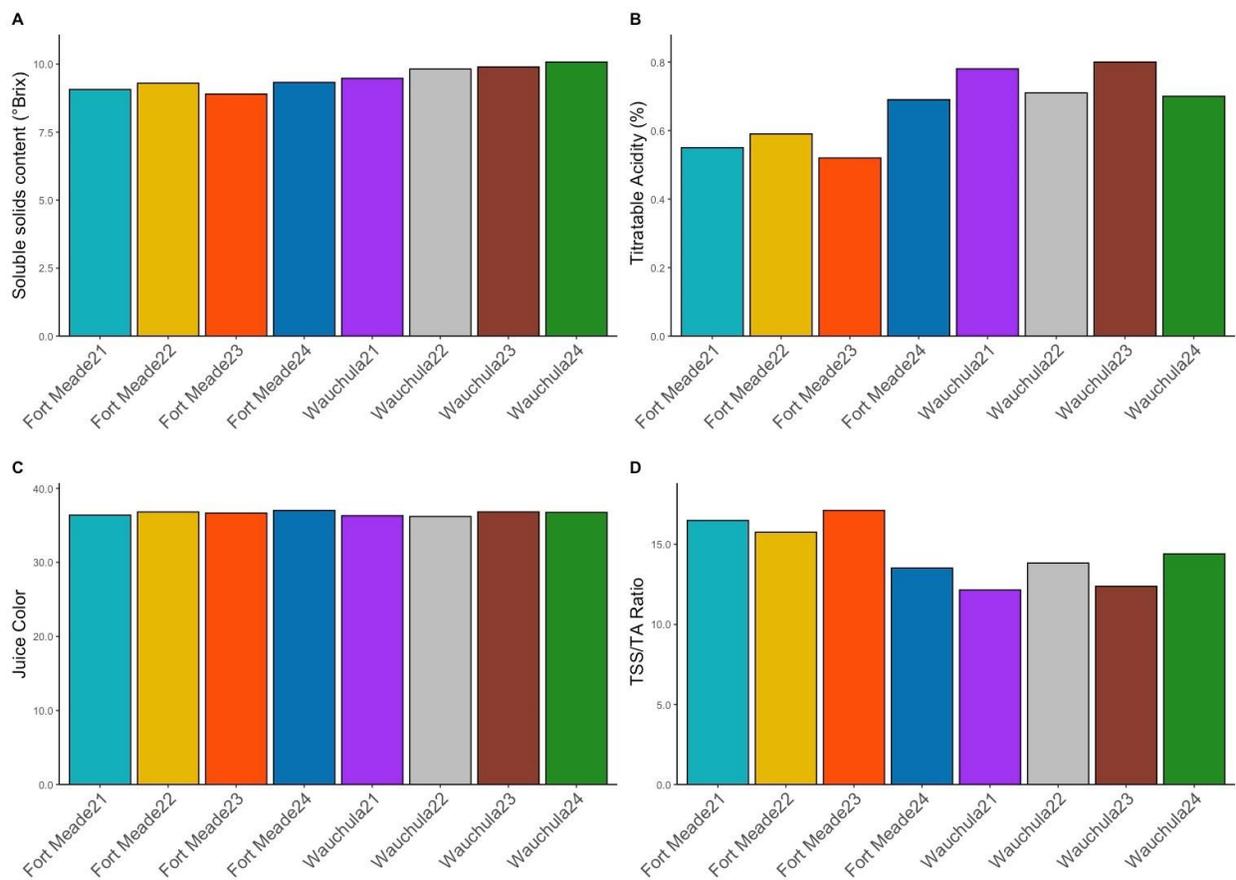

Fig 21. Fruit harvest in Florida.

Figure 22. Juice quality analysis for (A) Soluble solids content, (B) Titratable acidity, (C) Juice color, (D) Soluble solids/titrable acidity ratio (ripening index - RI), and for Wauchula and Fort Meade Valencia CitruSaver plots treated through micro jet irrigation.