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Field Performance of 'Valencia' Sweet Orange Trees Grafted onto Pummelo Interstocks and Swingle Citrumelo Rootstocks under Huanglongbing (HLB) Endemic Conditions

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Abstract: Interstocks have been used in fruit tree cultivation to regulate tree size and improve fruit production and quality. In this study, several Huanglongbing (HLB)-tolerant open-pollinated pummelo interstock candidates were evaluated as interstocks between the Swingle rootstock and the 'Valencia' scion, with Swingle serving as the control interstock. After 5 years in the field, most trees did not exhibit visual HLB symptoms, although the trees were infected with HLB, and the *Ca*Las Ct values in the 'Valencia' leaves of the different interstock treatments ranged between 25.88 and 27.82. Although the foliar chlorophyll content among the interstock treatments was not highly significant (p-value = 0.0313), the foliar starch content was significantly different (p-value = 0.0018). 'Valencia' grafted onto 5-1-99-3 and HBJL-4 interstocks (both open pollinated seedlings of the Hirado Buntan pummelo) exhibited the highest total phenolic compound (TPC) levels (46.44 and 46.36 mg gallic acid g^{-1} FW). Transcripts of *CsPR1* and *CsPR2*, two pathogenesis-related (PR) genes, were upregulated in 'Valencia' grafted onto open pollinated seedling selections of the red shaddock pummelo, Liang Ping Yau pummelo, and Hirado Buntan pummelo compared with 'Valencia' grafted onto Swingle. All interstocks influenced the tree growth rate and improved canopy volume in the field compared to the control trees without any interstocks (p-value = 0.0085). The 5-4-99-7 (red shaddock pummelo) and 8-1-99-1B (Liang Ping Yau pummelo) interstock trees had the highest canopy volume among all the treatments. We propose, based on our current results, that HLB-tolerant citrus accessions, when judiciously used as interstocks, may enhance plant defense and provide increased HLB tolerance to susceptible scions.

Keywords: citrus; interstocks; citrus greening; *Candidatus* Liberibacter asiaticus (*Ca*Las); antioxidants; pathogenesis-related proteins

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1. Introduction

Huanglongbing (HLB), caused by the phloem-limited bacterium 'Candidatus Liberibacter asiaticus' (*Ca*Las), affects citrus production in many countries, including the USA [1,2]. This disease causes massive economic losses and reduces the productivity of most commercially cultivated citrus varieties [3]. Some citrus varieties are more tolerant of *Ca*Las infection than others, indicating the potential to mitigate Huanglongbing disease [4–6]. Many strategies have been developed to withstand HLB stress, including conventional breeding of tolerant genotypes [7], generating transgenic germplasm possessing tolerance traits [8,9], application of antimicrobials such as antibiotics [10,11] and antimicrobial peptides [12], nutrition management [13], insect vector management [14], and thermotherapy [15].

All commercially cultivated citrus varieties are propagated by grafting onto selected rootstocks. The selection of these rootstocks depends on specific desirable traits. The most important factors that define successful rootstocks are (1) enhanced nutritional uptake, (2) soil adaptation, (3) tolerance to abiotic and/or biotic stressors, (4) yield improvement,

and (5) fruit quality enhancement. In Florida, Swingle and Kuharske/Carrizo have consistently been the most common rootstocks, even though they are HLB susceptible [16]. Under HLB endemic conditions, there is an urgent need to select new methods and germplasm to support scion growth, especially as many commercially important citrus scions are also susceptible to *Ca*Las infection. The interstock, which is a piece of graft-compatible plant tissue inserted between the rootstock and the scion, can be used as a "bridge" between the commercial scion and the rootstock. Interstocks affect tree production and longevity, improve fruit quality, control tree size [17], and enhance abiotic stress tolerance [18]. They are widely used in many fruit crops, such as lemon [19], apple [20,21], plum [22], and sweet cherry [23], to regulate tree size and improve fruit production and quality. Furthermore, the usage of interstock tissues can compensate for incompatibility between scion and rootstock, prevent tree decline following grafting, prevent bulge formation, avoid slow sap flow, and regulate growth. In such trees, owing to the active communication between the scion and the rootstock through the interstock, it is possible that an HLB-tolerant interstock may confer disease tolerance to the scion and the rootstock.

In the present study, we assessed field trees of 'Valencia' sweet orange grafted onto pummelo interstock and Swingle rootstock. To gain insights into their performance, we examined the chlorophyll content, starch content, total phenolic compounds, and DPPH radical scavenging activity in each combination of scion–interstock–rootstock. Furthermore, we investigated the differential expression of pathogenesis-related (PR) protein transcripts, specifically *CsPR1* and *CsPR2*. These analyses aimed to provide a comprehensive understanding of scion–interstock–rootstock interactions and their impact on physiological and molecular aspects of 'Valencia' sweet orange.

2. Materials and Methods

2.1. Plant Materials

The interstock candidates utilized in this study consisted of mature open-pollinated pummelo trees that were selected based upon their enhanced field tolerance to HLB. Six-inch interstock sticks were obtained from mature HLB tolerant field trees and grafted onto Swingle citrumelo. The selected pummelo trees used for interstock material were UKP-1 (seedling selection of unknown parentage), HBJL-1, HBJL-4, 5-1-99-2-S5 and 5-1-99-3 (seedling selections of Hirado Buntan pummelo; HBP), 5-4-99-3 and 5-4-99-7 (seedling selections of red shaddock pummelo), 7-2-99-11 (seedling selections of large pink pummelo), and 8-1-99-1B (seedling selections of Liang Ping Yau pummelo). The pummelo trees were selected for interstock use based on their enhanced HLB tolerance under field conditions. In this experiment, there were 10 replications from each scion–interstock–rootstock combination, and trees budded directly onto Swingle were used as the control. The interstock–swingle rootstock trees were subsequently cleft grafted with a field-derived HLB-infected 'Valencia' scion (Figure 1). Trees were maintained in the greenhouse and, after one year of successful grafting, trees were planted in the field in a randomized block design format. All trees were subsequently analyzed after 4 years in the field (5-year total age).

2.2. CaLas Assessment in 'Valencia' Leaves

*Ca*Las titers in the infected 'Valencia' leaves were evaluated from genomic DNA extracted from the leaf petioles and midveins of fully expanded leaf tissues using the GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) and normalized at 25 ng/µL. qPCR was performed using a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific) using TaqMan Gene Expression Master Mix and CQUL primers (Table 1) to amplify a *Ca*Las rplJ/rplL ribosomal protein gene [24]. The real-time qPCR Ct values $\leq$ 35 were assigned as positive for *Ca*Las 16 s rRNA infection, whereas real-time qPCR Ct values > 35 indicated negative.

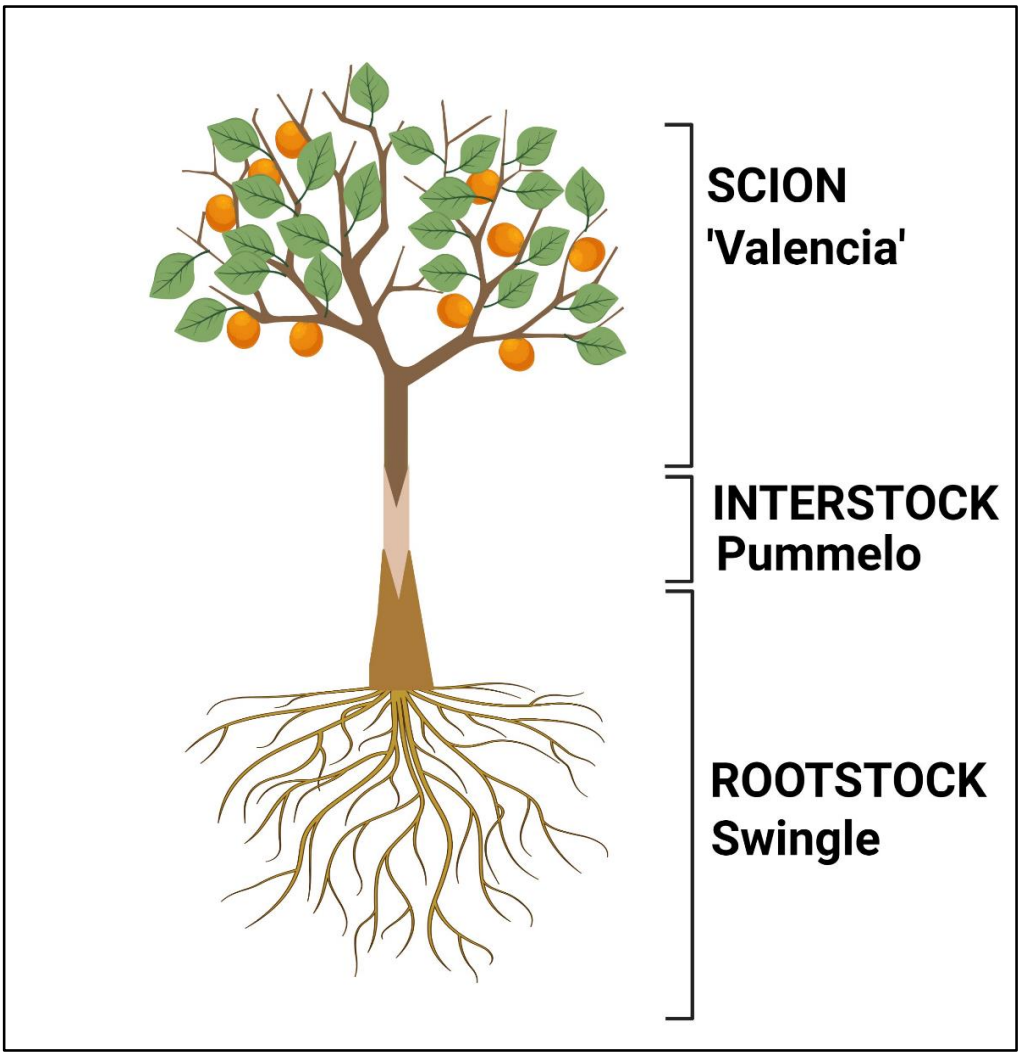

Figure 1. Schematic representation of the interstock grafting process.

Table 1. Primer sequences used to amplify an 87-bp fragment of the *Ca*Las rplJ/rplL ribosomal protein gene for SYBR Green-based real-time PCR assay.

Description (Gene Symbol)	Forward and Reverse Primer Sequences (5′ to 3′)
CQUL-F	TGGAGGTGTAAAAGTTGCCAAA
CQUL-R	CCAACGAAAAGATCAGATATTCCTCTA
CQUL-Probe	FAM-ATCGTCTCGTCAAGATTGCTATCCGTGATACTAG-IBFQ

2.3. Physiological and Biochemical Variables

Fifteen mature leaves were harvested from the trees during November, frozen, ground in liquid nitrogen, and kept at −20 °C for use in biochemical assays. Ten biological replicates were sampled from each interstock selection, and three technical replicates were sampled from each tree.

2.3.1. Foliar Photosynthetic Pigments and Starch Quantification

The chlorophyll pigments (chlorophyll a and b and total chlorophyll) were estimated according to Lichtenthaler and Wellburn [25]. Starch content was determined according to Rosales and Burns [26], with some modifications. Fresh tissues (100 mg) were homogenized in 700 µL of distilled water. Leaf samples and standard rice starch samples were boiled

in distilled water for 10 min and cooled in a water bath. Samples were centrifuged for 2 min at 6000 rpm, and the supernatant aliquots were collected into clean tubes. A 300 µL aliquot of supernatant was mixed with 900 µL of 100% ethanol, vortexed and centrifuged for 10 min at the maximum speed. One milliliter of distilled water was used to dissolve the pellet, and then fifty microliters of KI:I2 (8 mM:50 mM) was added. The color change was recorded at 594 nm in a spectrophotometer to quantify the starch content and compared with the color change of pure starch (Sigma Aldrich, St. Louis, MO, USA) as a standard.

2.3.2. Total Phenolic Compounds and DPPH Radical Scavenging Activity

Total phenolic compounds were estimated according to Singleton and Rossi [27]. In brief, 100 mg of fresh leaf was extracted in 1 mL of 80% ethanol and then centrifuged for 20 min at 10,000 rpm. The supernatant was incubated overnight at room temperature until evaporation and complete dryness. Distilled water (5 mL) was added to the plate to dissolve the dried gel. Two hundred microliter aliquots were diluted to 3 mL in distilled water. The folin reagent was added at 0.5 mL for 3 min, and then 2 mL of (20%) Na_2CO_3 solution was added to each tube. The color change was recorded after 60 min at 650 nm. The standard curve of phenol was prepared by measuring 1 mL of a series of ethanolic gallic solutions at different concentrations from 0 to 1.00 mg/mL. The phenol content was expressed as mg gallic acid per 100 g fresh weight tissue.

The DPPH free-radical scavenging activity was measured using the method described by Blois [28]. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) was prepared fresh at 1 mM solution in methanol. Equal amounts of DPPH solution and leaf extracts were mixed and incubated for 30 min in the dark. The absorbance was measured at 517 nm using a spectrophotometer, and methanol was used as a blank solution. The control solution was DPPH added to the methanol solution instead of the leaf extract. The analysis was performed in triplicate. DPPH inhibition was expressed as a percentage, as shown in the following equation:

$$\% \text{ DPPH inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

where A control is the mixture of methanol and DPPH solution and A sample is the mixture of sample extract and DPPH solution.

2.3.3. Tree Canopy

Canopy volume, expressed as cubic meters, was calculated as described by Vashisth and Grosser [29] using a geometric prolate spheroid formula: $[(4/3)\ (\pi)\ (H/2)\ (ACR)^2]$, where $\pi = 3.14$, H = tree height, and ACR = average canopy radius. ACR was calculated by dividing the tree diameter by 2 and calculating the average radius. The tree diameter was measured in two directions: east to west (D1) and north to south (D2). The percentage increase in canopy volume was calculated as the increase in canopy volume from the time of pruning until the end of the experiment.

2.4. Gene Expression Assessment

RNA was isolated from 100 mg of finely ground leaf tissues using a Direct-zol RNA Miniprep (Zymo Research, Irvine, CA, USA) kit according to the manufacturer's protocol. qPCR was performed with a final volume of 20 µL using PowerUp SYBR Green Master Mix (Thermo Fisher Scientific) according to the manufacturer's instructions. Each sample was tested twice in three replicates, and the data were analyzed using Applied Biosystems software version 3.0.1. The relative expression of the selected gene was calculated by the $2^{-\Delta\Delta CT}$ method [30]. The actin housekeeping gene was used as an endogenous control. The primer sequences of the genes evaluated in this study are outlined in Table 2.

Table 2. List of the primer sequences used in the SYBR Green-based real-time PCR assay.

Description (Gene Symbol)	Forward and Reverse Primer Sequences (5′ to 3′)
CsPR1	AACTCGCCTCAAGACTACCT CTGCAACTGTGTCGTTCCATA
CsPR2	ACTTCGCTCAGTACCTTGTTC GGCAGTGGAAACCTTGATTTG
β-actin	GCTGCCTGATGGCCAGATC AGTTGTAGGTAGTCTCATGAA

2.5. Statistical Analysis

Data were analyzed using one-way ANOVA in a completely randomized design with ten replications per treatment. The Tukey–Kramer HSD test was used post hoc to calculate significant differences between treatments. All statistical analyses were performed on JMP Pro version 16 (SAS Institute, Cary, NC, USA). Differences were significantly different when p values were less than 0.05%.

3. Results and Discussion

HLB is an often-fatal disease affecting the citrus industry worldwide. Although many strategies have been studied for HLB mitigation, most of the applied strategies have shown limited success in the field. Thus, breeding and developing tolerant cultivars may provide an effective solution and support long-term citriculture and combat HLB [31]. We selected several open-pollinated and HLB-tolerant pummelo trees that were originally derived from a large-scale screening population of zygotic pummelo seedlings in the late 1990s. The selections were based on their overall growth and vigor, even under endemic HLB conditions. Subsequently, they were used to improve the response to HLB when used as interstocks in this study.

3.1. CaLas Assessment in 'Valencia' Leaves

The trees were evaluated for the presence of *Ca*Las using qPCR. As expected, all trees were infected, and the *Ca*Las bacterial titer in 'Valencia' leaf petioles ranged between 25.88 and 27.82 in 'Valencia' grafted onto pummelo selections and 27.09 in 'Valencia' grafted onto Swingle (Table 3). These results indicate that *Ca*Las was able to multiply in all the different combinations, and there were no significant differences between the treatments. When 'Valencia' trees have low *Ca*Las Ct values [32], they usually exhibit severe symptoms of citrus greening disease, including stunted growth, yellow shoots, and blotchy mottled leaves. This was not observed in most of our interstock trees, even though they had been in the field for 4 years and were infected to begin with.

3.2. Physiological and Biochemical Variables

3.2.1. Foliar Photosynthetic Pigments and Starch Content

A significant difference in foliar chlorophyll content (p value = 0.0313) was observed when comparing the effects of different selections, as indicated in Table 4. The highest foliar chlorophyll content of 'Valencia' was recorded in the graft combinations with HBJL-1 and 8-1-99-1B, with values of 7.70 and 7.78 mg^{-1} g FW, respectively; however, 'Valencia' grafted onto Swingle recorded 6.71 mg^{-1} g FW. Additionally, a significant difference in foliar starch content (p value = 0.0018) was observed among the selections. A slight difference was observed in the content of foliar starch among the graft combinations. 'Valencia' grafted onto HBJL-4 and Swingle displayed the highest foliar starch content, with values of 34.50 and 38.22 $\mu g \cdot mm^{-2}$, respectively, whereas the lowest value (16.81 $\mu g \cdot mm^{-2}$) was recorded with 5-4-99-7 (Figure 2).

Table 3. Quantification of *Ca*Las bacterial titers following qPCR from leaf petiole of 'Valencia' sweet orange grafted into pummelo interstocks and Swingle rootstocks.

Interstocks	Ct Values of *Ca*Las
UKP-1	27.06 ± 0.34 [ab1]
HBJL-1	27.09 ± 0.07 [ab]
HBJL-4	25.88 ± 0.96 [b]
7-2-99-11	26.87 ± 0.95 [ab]
5-4-99-3	26.10 ± 0.20 [ab]
5-4-99-7 S2	26.15 ± 0.61 [b]
5-1-99-2	27.14 ± 0.12 [ab]
5-1-99-3	27.82 ± 0.46 [a]
8-1-99-1B	25.91 ± 0.57 [b]
Swingle	27.06 ± 0.49 [ab]
p value	NS

[1] Tukey–Kramer HSD test was used post hoc to calculate significant differences between treatments. Means followed by the same letter were not significantly different at $p < 0.05$. NS: not significant.

Table 4. Foliar chlorophyll content of 'Valencia' sweet orange grafted into pummelo interstocks and Swingle rootstocks.

Selection	Chlorophyll a mg^{-1} g FW	Chlorophyll b mg^{-1} g FW	Total Chlorophyll mg^{-1} g FW
UKP-1	3.68 [a,1]	2.76 [ab]	6.44 [c]
HBJL-1	4.47 [a]	3.22 [a]	7.70 [a]
HBJL-4	4.07 [a]	2.57 [b]	6.65 [bc]
5-1-99-2 S5	4.19 [a]	3.04 [ab]	7.23 [abc]
5-1-99-3	4.09 [a]	3.03 [ab]	7.13 [abc]
5-4-99-3	4.61 [a]	2.89 [ab]	7.50 [ab]
5-4-99-7	3.90 [a]	2.80 [ab]	6.74 [bc]
7-2-99-11	4.44 [a]	3.07 [ab]	7.51 [ab]
8-1-99-1B	4.50 [a]	3.28 [a]	7.78 [a]
Swingle	4.61 a	2.84 [ab]	6.71 [bc]
p value	NS	0.0057 *	0.0313 *

[1] Tukey–Kramer HSD test was used post hoc to calculate significant differences between treatments. Means followed by the same letter were not significantly different at $p < 0.05$. NS: Not Significant, * Represents significant difference between the means.

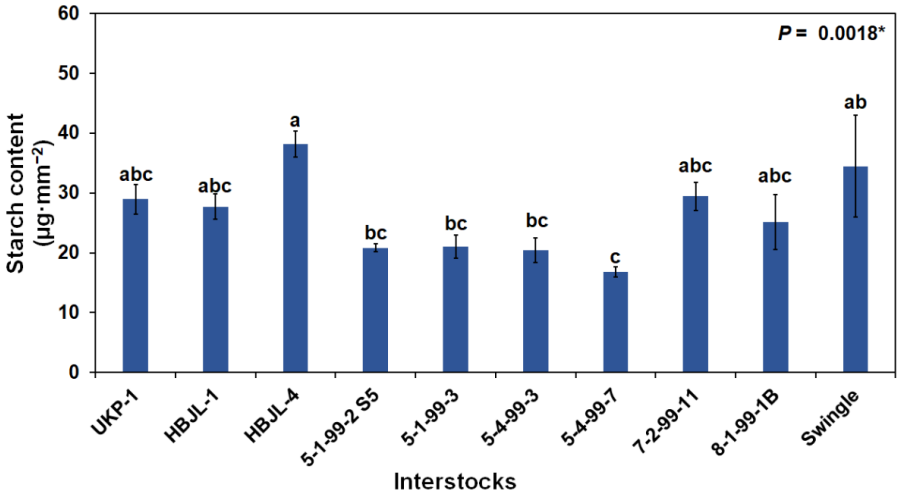

Figure 2. Foliar starch content of 'Valencia' sweet orange grafted into pummelo interstocks and Swingle rootstocks. Means compared using Tukey–Kramer HSD test. Means followed by the same letter were not significantly different at $p < 0.05$. * Represents significant difference between the means. The error bars indicate standard error (SE; $n = 10$).

The significant difference in the foliar starch content indicates the importance of using the interstock in the citrus tree structure. Starch accumulation in photosynthetic cells, vascular parenchyma, and phloem elements can be used as an indicator for disorder in carbohydrate metabolism and HLB infection in citrus [33]. It causes phloem blockage and limited sugar export in *Ca*Las-infected tissues [34]. The differences in starch content and other physiological parameters indicated the superiority of pummelo interstocks when compared with trees that were grown without an interstock and grafted directly onto Swingle.

3.2.2. Total Phenolic Compounds and DPPH Radical Scavenging Activity

The 'Valencia' scion foliar TPC content was significantly different (p value < 0.0001) when the effect of the different selections was compared (Figure 3A). 'Valencia' grafted onto 5-1-99-3 and HBJL-4 interstocks exhibited the highest TPC values (46.44 and 46.362 mg gallic acid g^{-1} FW), whereas 'Valencia' grafted onto Swingle recorded 37.70 mg gallic acid g^{-1} FW. The DPPH free-radical scavenging activity of 'Valencia' leaves showed a significant difference between the selections (p value = 0.0013). 'Valencia' grafted onto 5-1-99-2 S5 recorded the highest DPPH content (90.35%) (Figure 3B).

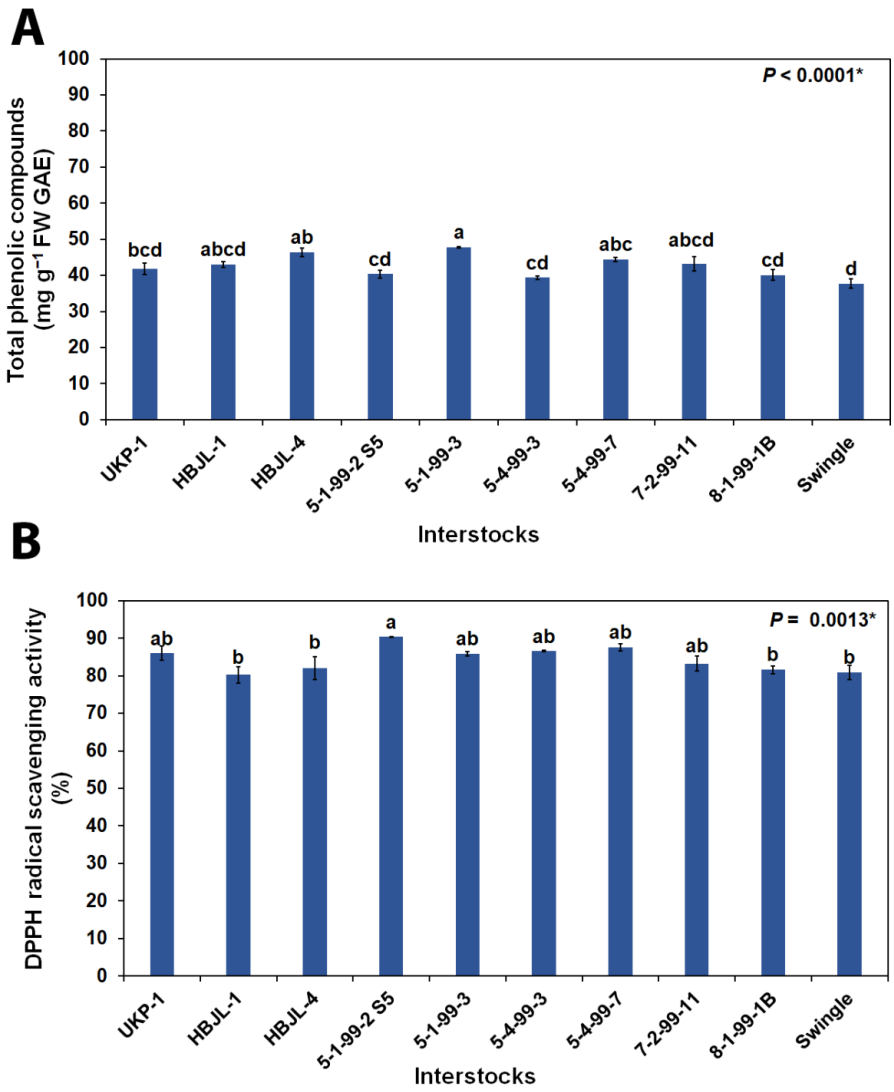

Figure 3. Foliar total phenolic compounds (**A**) and DPPH radical scavenging activity (**B**) of 'Valencia' sweet orange grafted into pummelo interstocks and Swingle rootstocks. Means compared using Tukey–Kramer HSD test; means followed by the same letter were not significantly different at $p < 0.05$. * Represents significant difference between the means. The error bars indicate standard error (SE; $n = 10$).

Recently, Weber et al. [6] reported that citrus greening disease (HLB) can be controlled by ROS detoxification via induction of antioxidant pathways. To mitigate the harmful effects of ROS, complex mechanisms have evolved in plants. These mechanisms include scavenging ROS via the accumulation of various enzymatic and nonenzymatic antioxidants [35]. Phenolic compounds are important antioxidants that play essential roles as antimicrobial agents in response to *Ca*Las [36] and Ca. Liberibacter solanacearum [37,38], and in response to abiotic stress [34,39,40].

The levels of phenolic compounds have been found to be affected by rootstocks [36]. Killiny et al. [36] recorded higher quinic acid in 'Sugar Belle' grafted onto Carrizo citrange or Swingle citrumelo compared to UFR-15 and higher ferulic acid in 'Sugar Belle' grafted onto 'Swingle'. The same study suggested that Swingle would be the optimal rootstock to enhance disease resistance based on its phenolic content. The current study indicated that using tolerant pummelo interstocks increased the total phenolic compounds in 'Valencia' leaves compared with those grafted onto Swingle.

The enhanced DPPH free-radical scavenging activity in 'Valencia' leaves indicated the capability of 'Valencia' grafted onto pummelo interstocks to mitigate the adverse effect of ROS compared with 'Valencia' grafted onto Swingle, but all treatments performed equally well, and there was no significant difference.

3.3. Tree Canopy

HLB-infected trees generally have poor tree canopy. In our study, all interstocks influenced the growth rate of tree canopy volume in the field (p value = 0.0085; Figure 4). The canopy volume measurements revealed that 5-4-99-7 exhibited the highest volume, reaching 56.97 m^3. This was followed by 8-1-99-1B and 5-1-99-2 S5, which recorded volumes of 38.98 and 37.98 m^3, respectively. In comparison, 'Valencia' grafted onto Swingle controls displayed a canopy volume of 16.39 m^3. Interstocks are widely used in fruit tree production. The influence of interstock on the vigor of cultivars depends on several factors. Genetic and agronomic factors are the main effective factors [20]. The decrease in growth induced by interstocks depends on the vigor of the interstock [41] and the selected rootstock and cultivar [42,43]. HLB-tolerant interstocks in this study (5-year-old trees) resulted in vigorous trees, which depended on the interstock used (Figure 5). Interstocks can be used for tree-size control in 'Minneola' tangelo and 'Valencia' [41]. Dutt et al. [44] reported that the architecture of sweet orange trees is modulated by endogenous hormonal and genetic alterations. Auxins, cytokinins, and gibberellin have a role in the regulation of plant vigor [44,45]. Many studies have reported that rootstocks can control tree size by modulating auxin and gibberellin (GA) signaling [46]. It remains unknown whether older interstock trees will continue to have good canopy density or whether there will be a decline as HLB symptomology increases.

3.4. Gene Expression of Pathogenesis-Related (PR1 and PR2) Genes

CsPR1 and *CsPR2* are induced following *Ca*Las infection [7–9,35]. The differential expression of these two pathogenesis-related genes (*CsPR1* and *CsPR2*) was investigated in 'Valencia' leaves. Our results showed a significant difference in the two tested genes (Figure 6). Five pummelo hybrids showed high expression of *CsPR1* and *CsPR2*. The expression of *CsPR1* genes was upregulated in 'Valencia' grafted onto 8-1-99-1B, followed by UKP-1, HBJL-4, and 5-4-99-7 (Figure 6A). *CsPR2* was upregulated in 'Valencia' grafted onto HBJL-4, 8-1-99-1B, UKP-1, 5-4-99-7, and HBJL-1 following *Ca*Las infection (Figure 6B).

Pathogenesis-related (PR) proteins are significant key molecules induced by phytopathogens as well as defense-related innate immune systems, especially systemic acquired resistance (SAR) [47]. Following pathogen attack, plants activate defense signaling pathways involving salicylic acid (SA) and jasmonic acid (JA), which further enhance PR protein accumulation, ultimately minimizing pathogen infection [48]. Dutt et al. [7] observed enhanced expression of *CsPR1* and *CsPR2* genes in the mesophyll of HLB-tolerant finger lime (*Citrus australasica*). Weber et al. [35] reported the presence of PR-1 proteins

related to a superfamily of secretory proteins rich in cysteine in HLB-tolerant finger lime. In the present study, 'Valencia' grafted onto pummelo interstocks was grown under HLB-endemic conditions, and enhanced *CsPR1* and *CsPR2* gene expression may have played a role in enhanced tolerance to HLB.

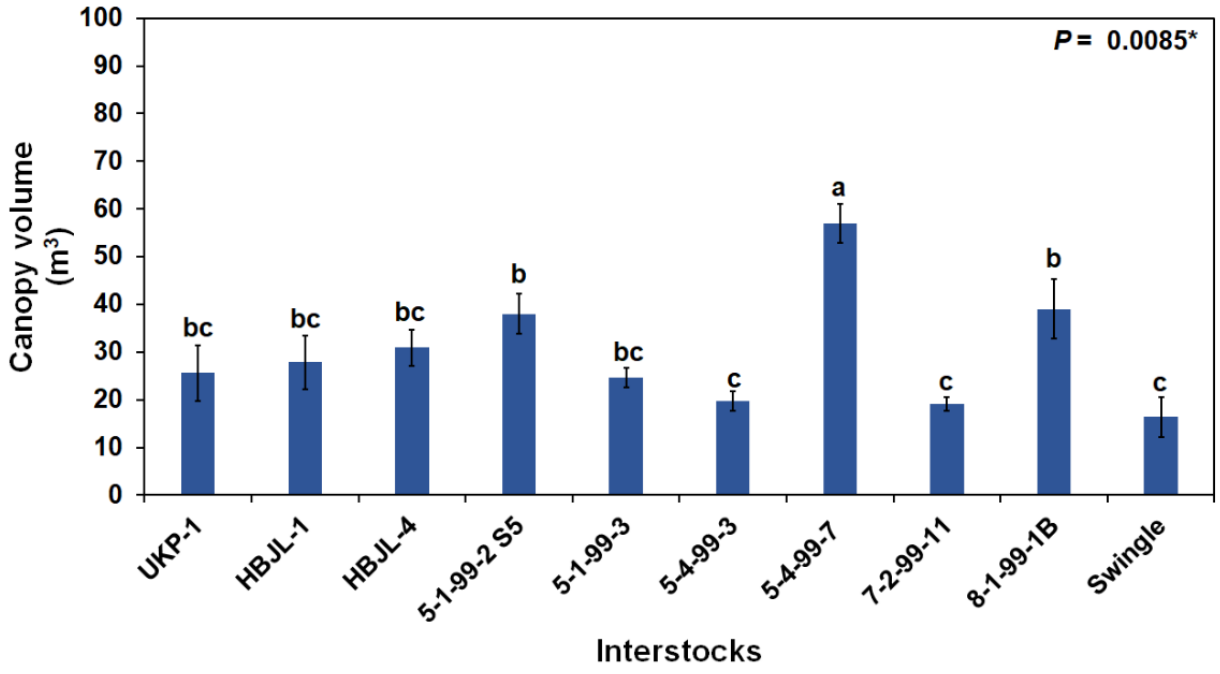

Figure 4. Canopy volume of 'Valencia' sweet orange grafted onto pummelo interstocks and Swingle rootstocks. Means compared using Tukey–Kramer HSD test; means followed by the same letter were not significantly different at $p < 0.05$. * Represents significant difference between the means. The error bars indicate standard error (SE; $n = 10$).

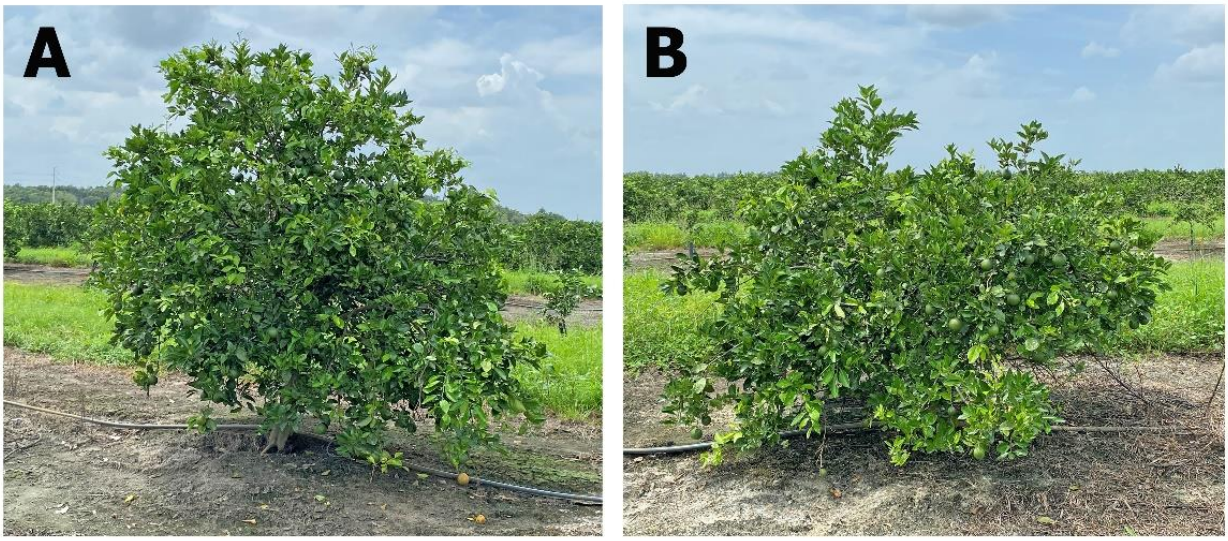

Figure 5. (**A**) "Valencia" sweet orange tree on 5-4-99-7 interstock (**B**) "Valencia" sweet orange tree on Swingle interstock.

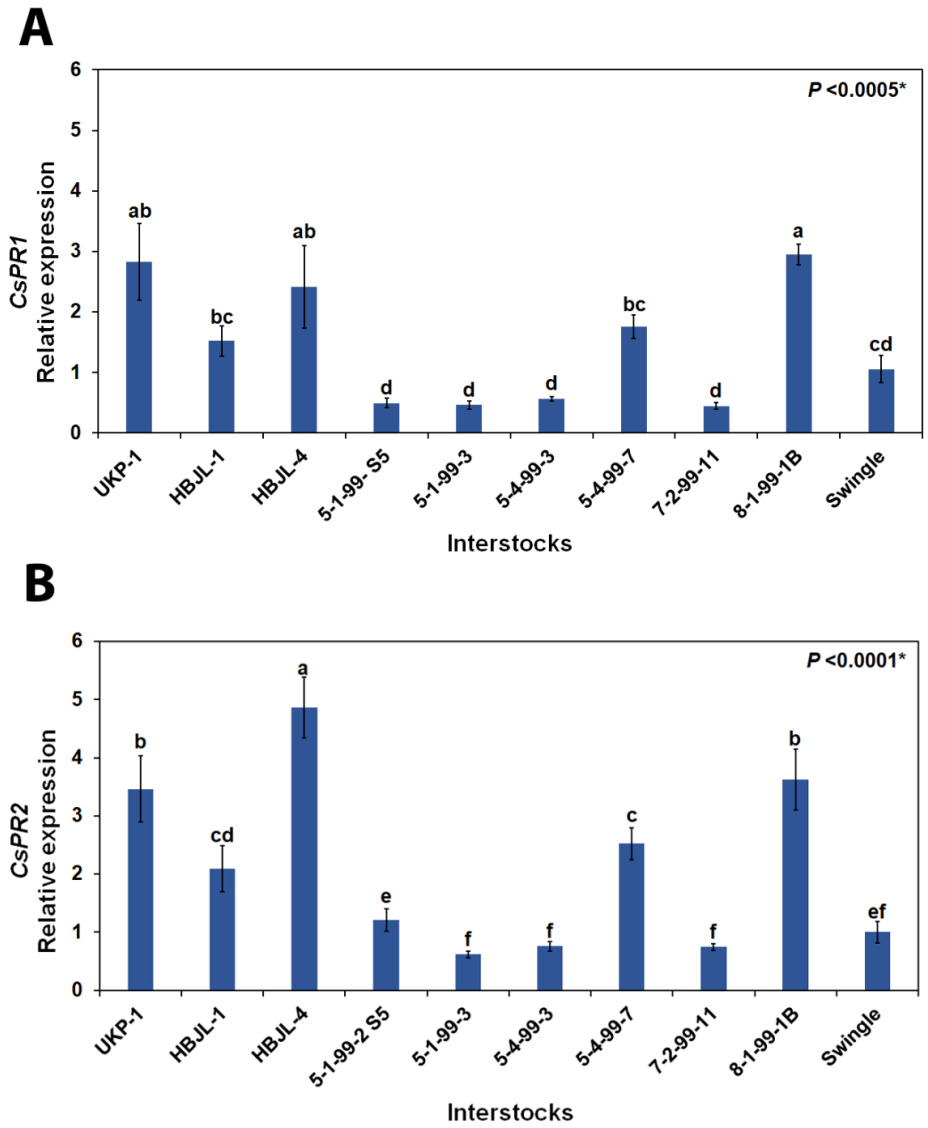

Figure 6. The differential expression of *CsPR1* (**A**) and *CsPR2* (**B**) transcripts of 'Valencia' sweet orange grafted into pummelo interstocks and Swingle rootstocks. Means compared using Tukey–Kramer HSD test; means followed by the same letter were not significantly different at $p < 0.05$. * Represents significant difference between the means. The error bars indicate standard error (SE; $n = 10$).

4. Conclusions

In this study, we provide evidence that HLB-tolerant pummelo interstocks can be used to produce vigorous trees and result in enhanced tolerance to susceptible scions. Although it was beyond the scope of the current study to understand the detailed biochemical mechanism occurring in the phloem, there was a definite interaction occurring in the phloem. The interstock had a pronounced effect over the Swingle control, although there was no difference in the *Ca*Las bacterial titer among the different treatments. Using an interstock can raise tree production costs and turnaround time but may allow citrus growers with another option to grow a productive tree even under endemic HLB conditions. Apart from the effect of using tolerant interstocks to enhance plant defense, interstocks can also be used to control citrus tree size and lead to either high-density plantings with dwarf trees or vigorous trees with wider spacing in the field.

Author Contributions: Conceptualization, M.D., J.W.G. and L.M.M.; methodology, M.D., J.W.G. and L.M.M.; software, L.M.M.; validation, M.D. and L.M.M.; formal analysis, M.D. and L.M.M.; investigation, M.D. and L.M.M.; resources, M.D. and J.W.G.; data curation, M.D. and L.M.M.; writing—original draft preparation, M.D. and L.M.M.; supervision and project administration, M.D. and J.W.G. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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