Grapefruit Production in Open Hydroponics System

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Abstract: Conventionally managed citrus orchards can be modified to incorporate advanced horticultural practices such as higher plant density and efficient water and fertilizer application known as open hydroponics system (OHS) to increase productivity under Huanglongbing (HLB) endemic conditions. A field study was conducted from 2013 to 2018 to evaluate the effect of an OHS on “Ray Ruby” grapefruit (RR) production under HLB-endemic conditions. We tested a combination of different rootstocks [Sour orange (RR/SO) and US-897 (RR/897)], tree planting densities [standard (STD, 358 trees per ha) and high density staggered (HDS, 953 trees per ha)], fertilization methods (dry granular—dry and fertigation—fert), and irrigation systems (double driplines—DD and microsprinkler—MS), arranged in five treatments: RR/SO_STD_dry_MS, RR/SO_HDS_fert_DD, RR/897_HDS_fert_MS, RR/897_HDS_fert_DD, and RR/SO_HDS_fert_MS. All trees were infected by Candidatus Liberibacter asiaticus five years after planting. Trunk diameter and canopy volume increased over time and were higher under RR/SO_STD_dry_MS compared to other treatments. Total fruit number increased in 2016/17 compared to other seasons; however, 65% of fruit were classified as small (<100 mm). Fruit produced under RR/897_HDS_fert_DD had the highest amount (79%) of adequate size fruit (100–117 mm) compared to other treatments. Fruit yield was similar for both rootstocks planted at HDS using DD and MS fertigation, and 67% higher than the standard treatment (RR/SO_STD_dry_MS). Soluble solid contents (SSC), titratable acidity, and SSC-to-titratable acidity ratio were not affected by the treatments. HDS planting resulted in higher fruit yield, irrespective of rootstock and irrigation system, representing an important advance in grapefruit production. Overall, our results demonstrated that production of grapefruit in high-density using OHS can be used by citrus growers who aim to make the best water and fertilizer management under HLB-endemic conditions.

Keywords: Citrus paradisi; citrus nutrition; irrigation management; fruit yield; fruit quality; nutrient use efficiency

1. Introduction

Citrus (Citrus spp.) is Florida’s most important agricultural commodity. Growers produce citrus for different markets, and focus mainly on round oranges (C. sinensis) for processed juice, with a small percent of navels, mandarins (C. reticulata), grapefruit (C. paradisi) and lemons (C. limon) for the fresh fruit industry, and lemons for extracting peel oil for processing. The U.S. citrus production for the 2018/19 season totaled 7.94 million tons, with Florida contributing with 44%. While economic data for the 2018/19 citrus season improved from the previous seasons, the improvement lagged expectations. Commercial citrus acreage and trees continued a decades-long decline, falling to its lowest value (174,258 hectares) in the 2018/19 season since the U.S. Department of Agriculture (USDA) began...
the current inventory in 1966. Total grapefruit produced in Florida decreased even more drastically from 40.9 million boxes in 2003/04 to 4.51 million in 2018/19. Excluding season 2004/05 impacted by hurricanes, Florida’s average grapefruit yield has rapidly declined from 52 t ha\(^{-1}\) in 2000/01 to only 19 t ha\(^{-1}\) in 2018/19 [1].

This decline is largely caused by Huanglongbing (HLB), a disease associated with *Candidatus Liberibacter asiaticus* (CLas), in addition to citrus canker (*Xanthomonas axonopodis* pv. *citri*), urban development, and recent hurricanes [2–4]. HLB is the most severe citrus disease, negatively impacting the citrus industry worldwide [5]. Once citrus is affected by HLB, it gradually becomes less productive. HLB alters plant physiological and morphological functioning, reducing photosynthesis and xylem flux, phloem transport, root length and density, which consequently affects nutrient uptake, translocation, and utilization [6]. Trees under HLB-endemic conditions have higher production costs and lower fruit yield and require enhanced horticultural practices to optimize orchard productivity [7]. In addition, several studies have shown that limited water resources coupled to environmental concerns also create a need for more efficient management practices [8].

New concepts for advanced citrus production systems (ACPS) and/or the open hydroponics system (OHS) have been investigated in recent years to maintain higher levels of productivity through improved water and fertilizer use efficiencies [8–10]. The combination of these practices makes citrus production more efficient and economically competitive. OHS refers to a set of management practices based on continuous nutrient application by fertigation using drip irrigation, resulting in a small wetted zone maintained near field capacity [11,12]. The use of OHS in citrus production may be an effective strategy for improving water and fertilizer use efficiency and uptake during critical phenological stages, possibly leading to higher fruit yield and more vigorous tree growth relative to conventional water and nutrient management practices. Growers using OHS can also easily control fertigation nutrient concentrations, modify water pH, reduce nutrient leaching, and improve fruit yield [11,13]. With enhanced water and nutrition management, trees can reach maturity faster, and continue growing canopy and producing fruit in the presence of HLB [12].

Additional horticultural practices have also been tested to increase fruit yield under high HLB pressure. New groves should be established using more trees per area to maximize productivity [10,14]. Some recent studies with high-density plantings showed promising results for HLB-affected trees [15–17]. Rootstock selection is also a critical factor to consider when modifying grapefruit orchard architecture and design [18].

The combination of OHS with high-density tree planting and productive rootstocks is being promoted under the name ACPS [8,9,19]. At present, the benefits of using OHS with high-density tree planting and HLB-tolerant rootstocks has not been investigated in grapefruit production in the Indian River District. Under current HLB-endemic conditions in Florida, OHS may be an effective strategy for accelerating tree growth rates in young orchards, resulting in earlier fruit production compared to conventional management practices [12]. Consequently, the validation of these principles and the assessment of their impact in HLB-affected grapefruit is necessary.

We hypothesize that higher tree density coupled with drip irrigation and more frequent fertigation can lead to higher grapefruit production. The objective of this study was to evaluate the effect of an OHS on grapefruit production under HLB-endemic conditions. The system was a combination of tree planting density, fertilization methods, and irrigation systems. We monitored the concentration of CLas DNA on plant leaf tissue, tree size, leaf nutrient concentrations, number of fruit, fruit yield, and fruit quality.

2. Materials and Methods

2.1. Experimental Area

A large-scale field study was conducted from September 2013 to February 2018 at the University of Florida (UF)/Institute of Food and Agricultural Sciences (IFAS) Indian River Research and Education
Center in Fort Pierce, Florida, USA (27°26’01.8” N, 80°26’49.80” W, and elevation 10 m). The soil of the experimental area was a Pineda soil series, classified as loamy, siliceous, and active hyperthermic Arenic Glossaqualfs according to USDA [20]. The land has a slope of 0% to 2% with poorly drained soil containing 96% sand, 2.5% silt, and 1.5% clay with 5.0 to 20 g kg$^{-1}$ organic matter [21]. We characterized the soil after planting in June 2014 to adjust initial fertilization by taking soil samples from 0–30 cm to determine extractable P, K, Ca, Mg, Mn, and Zn by Mehlich-3 extraction along with pH$_{\text{water}}$ and the cation-exchange capacity (CEC). Samples were processed in a commercial soil analysis lab (Waters Agricultural Laboratories Inc., Camila, GA, USA). The results indicated P = 7 mg kg$^{-1}$, K = 14 mg kg$^{-1}$, Ca = 557 mg kg$^{-1}$, Mg = 37 mg kg$^{-1}$, Mn = 1 mg kg$^{-1}$, Zn = 2 mg kg$^{-1}$, pH = 5.9, and CEC = 4 cmol$_c$ kg$^{-1}$. Poor drainage in coastal flatwood soils requires construction of elevated cultivation beds. Many areas that correspond to Pineda soil are currently used for grapefruit production.

The experimental site is characterized by a humid subtropical climate. Weather data was obtained from the Florida Automated Weather Network (FAWN) during the study (Figure 1).

![Figure 1](image-url)

**Figure 1.** Monthly reference evapotranspiration (ET$_0$) (a), maximum, minimum, and average air temperatures at 2 m above the soil surface, rainfall precipitation and air relative humidity in 2014 (b), 2015 (c), 2016 (d), and 2017 (e). Data were collected from on-site weather stations managed by the Florida Automated Weather Network (FAWN).
2.2. Experimental Design and Treatments

The experiment was arranged in a randomized complete block design with five replications. We tested a combination of rootstocks: Sour Orange (C. aurantium) and US-897 [Cleopatra (C. reticulata) × Flying Dragon (Poncirus trifoliata)], tree planting densities [standard and high-density staggered planting (HDS)], fertilization methods (dry granular and water-soluble fertilizer), and irrigation systems (drip and microsprinkler), arranged in five treatments:

• RR/SO_STD_dry_MS = “Ray Ruby” grapefruit (RR) on Sour Orange (SO) rootstock + standard spacing (STD, 3.8 × 7 m, 358 trees per ha) + 12N-1.31P-7.47K controlled-release fertilizer (CRF) applied in-ground + microsprinkler (MS) irrigation (one emitter per tree; blue microsprinklers—40.5 LPH at 138 kPa).
• RR/SO_HDS_fert_DD = “Ray Ruby” grapefruit on Sour Orange rootstock + HDS [(2.74 × 1.5 × 0.9 m) × 6.1 m, 953 trees per ha] + 15N-2.6P-22.4K water-soluble fertilizer applied by fertigation + drip irrigation (four emitters per tree, installed on double rows; blue dripper—3.8 LPH at 138 kPa).
• RR/897_HDS_fert_MS = “Ray Ruby” grapefruit on US-897 rootstock + HDS + 15N-2.6P-22.4K applied by fertigation + microsprinkler irrigation (same as above).
• RR/897_HDS_fert_DD = “Ray Ruby” grapefruit on US-897 rootstock + HDS + 15N-2.6P-22.4K applied by fertigation + drip irrigation (same as above).
• RR/SO_HDS_fert_MS = “Ray Ruby” grapefruit on Sour Orange rootstock + HDS + 15N-2.6P-22.4K applied by fertigation + microsprinkler irrigation (same as above).

2.3. Treatments Application and Cultural Practices

In September 2013, one-year-old “Ray Ruby” grapefruit trees budded on Sour Orange and US-897 rootstock were planted on raised beds (two rows per bed). We tested these rootstocks based on tolerance to environmental stresses, including pests and low temperatures, and the effect on the vigor, health, productivity of the tree as well as fruit quality. In addition, the rootstock Sour Orange induces high yield and excellent fruit quality, and is considered moderately tolerant to HLB [22]. The interest in US-897 remains especially strong because of the dwarfing effect of this rootstock and suitability for high-density plantings and intensive tree management [23].

Fertilizer rates were based on recommendations for grapefruit in Florida provided by Morgan and Kadyampakeni [21] for trees from 4 through to 7 years of age at 180 kg ha⁻¹ of N per year. Tree planting density dictated the fertilizer source and application method used. At standard tree density (358 trees per ha), we used a 12-3-9 (12N-1.31P-7.47K) granular CRF (Harrell’s LLC, Lakeland, FL, USA) in which 100% of N and P and 95% of K were available as CRF. Iron was in chelate form, while all other micronutrients were as sulfur-coated products. We applied CRF by hand within the dripline at an annual rate of 180 kg ha⁻¹ of N in three split applications in February, July, and October. Fertigation supplied nutrients to trees in high-density staggered plantings (953 trees per ha). We used a 15-6-27 (12N-2.62P-22.31K) water-soluble fertilizer (Agrolution® pHLow; ICL, Summerville, SC, USA) and applied it weekly at an annual rate of 180 kg ha⁻¹ of N to high-density staggered trees.

Irrigation systems varied by planting arrangement. At standard tree density, each tree was irrigated by one microsprinkler emitter (Fan-Jet® PLUS; Bowsmith, Exeter, CA, USA) with a 40.5 L h⁻¹ water discharge at 138 kPa, 4.8 m diameter wetted pattern, and an irrigation efficiency of 93% measured in 2017 by the FDACS Mobile Irrigation Labs [24]. Microsprinkler irrigation was also used at the same rate, pressure, and wetted pattern with one emitter used to irrigate two trees, and an irrigation efficiency of 92%. Microsprinklers for both planting arrangements were placed approximately 15 cm perpendicular to trees. The remaining high-density staggered trees were under drip irrigation (Jain Irrigation Inc., Fresno, CA, USA). We spaced two drip lines at 30 cm from each row. Drippers discharged 3.8 L h⁻¹ at 138 kPa and were spaced 30 cm apart, resulting in a total of four emitters per tree, and an irrigation efficiency of 93%. Both microsprinkler and drip irrigation were applied daily to match estimated crop evapotranspiration (ETc) as indicated by Morgan and Kadyampakeni [21] from non-bearing
(years 2013–2016) and bearing trees (>2017). ETc was monitored from 2013–2017 using a local weather station (ET Sensor; Hunter Industries, San Marcos, CA) and after 2017 using the Florida Automated Weather Network (FAWN) station located in St. Lucie West, Florida.

Pest, disease and weed control were performed in accordance with the UF/IFAS best management practices [21].

2.4. Measurements

We determined the concentration of CLas DNA in plant leaf tissue, tree size, leaf macro and micronutrient concentrations, total number of fruit, fruit size, fruit yield, and fruit quality parameters (SSC, acidity, and SSC-to-acidity ratio).

2.4.1. Concentration of CLas DNA in Plant Leaf Tissue

Leaves for CLas DNA determination were collected annually. A total of six fully expanded mature leaves were taken from summer flush in all four cardinal sections per tree. The petiole and midribs were used for CLas detection by real-time quantitative polymerase chain reaction (qPCR) [25]. Trees were considered positive for CLas when normalized CtCLas values were ≤32 [26].

2.4.2. Tree Size

Tree size was assessed annually in December before fruit harvest by measuring tree size, trunk diameter and canopy width. Tree height was measured from the soil surface to the top of the canopy. Trunk diameter was measured using a digital caliper 8 cm above the graft union. Canopy width was measured as mean canopy radius (MCR) in north-south and east-west directions. Canopy volume was calculated using Equation (1) [27].

\[
\text{Canopy volume (m}^3) = \left( \frac{4}{3} \right) \pi \left( \frac{H}{2} \right)^2 (MCR)^2
\]

where \(H\) is tree height (m) and \(MCR\) is mean canopy radius (m).

2.4.3. Foliar Nutrient Concentration

Leaf samples were collected annually (2014–2017) in August (on spring flush) to determine nutrient concentrations according to Morgan and Kadyampakeni [21]. Briefly, 20–30 fully expanded mature leaves were collected from each plot in different parts of the tree. Samples were preserved in a cooler during the sampling period and were acid-washed prior to analysis. Leaf tissue samples were dried for 72 h at 65 °C, ground to pass a 1 mm screen, and analyzed by inductively coupled plasma atomic emission spectroscopy (ICP–AES) to determine the concentration of P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn. Foliar N concentration was measured by macro dry combustion using an elemental analyzer (LECO CNS-2000; LECO Corporation, St. Joseph, MI, USA). Foliar nutrient concentrations were compared with optimum levels for Florida citrus [28].

2.4.4. Number of Fruit, Fruit Size, and Fruit Yield

Number of fruit, fruit size, and fruit yield data were collected from 2015/16 to 2017/18 harvesting seasons. Total number of fruit and fruit diameter were obtained by harvesting all fruit and passing them through an optical sorter (Autoline, Reedley, CA, USA). Tiny, diseased, and damaged fruit were discarded. The sorter was programmed to categorize fruit size into the following count categories: >21 fruit per carton (>123 mm), 27 (123–117 mm), 32 (117–111 mm), 36 (111–105 mm), 40 (105–100 mm), 48 (100–95 mm), 56 (95–90 mm), 64 (90–84 mm), and <64 (<84 mm). Fruit diameter was calculated as: (average diameter in each fruit count category \(\times\) number of fruit on that category) \(÷\) total fruit count. Measurements were converted into number of fruit per carton within three categories (small ≤ 100 mm; adequate = 100–117 mm; and large > 117 mm) [29].
The harvested fruit were weighed and recorded separately from each tree using a portable digital scale (D51P60HR1; Ohaus, Los Angeles, CA, USA). Total fruit yield was obtained from the weight of all fruit harvested from each plot and extrapolating fruit yield per plot to a hectare basis.

2.4.5. Fruit Quality

Samples of 20 fruit randomly chosen from trees within each experimental unit were collected for fruit quality analysis in 2016/17 and 2017/18. Fruit were squeezed with a juicer (model 2702; Brown International Corporation, Covina, CA, USA), and juice was weighed. Soluble solids content (SSC) was determined using a temperature-compensated, digital refractometer (HI96801; Hannah Instruments, Woonsocket, RI, USA). Yield of solids was determined by multiplying juice yield by SSC as described by Wardowski et al. [30]. Titratable acidity was determined by titrating 40 mL of juice to pH 8.3 with 0.3125 N sodium hydroxide (NaOH; Sigma-Aldrich, St. Louis, MO, USA) using an automatic potentiometric titrator (HI931; Hanna Instruments Inc., Woonsocket, RI, USA) and expressed as percentage (%) anhydrous citric acid [31]. Fruit quality was also expressed as SSC-to-titratable acidity ratio (SSC divided by titratable acidity).

2.5. Statistical Analysis

Data were analyzed for normality (Proc UNIVARIATE), analysis of variance (ANOVA) (Proc GLM), and Tukey’s multiple comparisons test (Proc LSMEANS) using SAS [32]. Treatments were treated as fixed effects, while replication was a random effect. Data were analyzed separately by year and considered statistically significant when probability (p) ≤ 0.05.

3. Results and Discussion

3.1. CLas Infection

Trees were monitored for the presence of CLas; if Ct values were lower than 32 in the qPCR test, trees were considered positive [26,33]. Infection by CLas increased over time, reaching 100% five years after planting (Figure 2). A similar effect was found in an orchard cultivated with “Valencia” sweet orange in a commercial grove in Immokalee, FL [34]. These authors reported that 83% of trees were positive for CLas in 2010, 88% in 2011, 99% in 2012, and 98% in 2014. These results indicate how quickly CLas can spread in Florida.

Infection by CLas was treatment-dependent. From 2014 to 2017, RR/SO_STD_dry_MS had higher Ct values and lower CLas infection (Figure 2a–d). However, in 2018, the entire orchard was infected (Figure 2e), with visible symptoms of HLB such as foliage loss, fruit drop, and tree death. The disease is severe, and other symptoms have also been reported, such as a few deformed and small-sized fruit [35], interveinal chlorosis of young leaves, chlorotic mottling of older leaves, moderate leaf drop, some stem dieback [34], loss of fibrous roots and depletion of starch in the roots [36]. Moreover, HLB-induced reduction in root density reduces water and nutrient uptake [37–39], causing whole tree decline that is often lethal [40,41].

Our study indicated that even with the increase in tree planting density, the use of different rootstocks, fertilization management, and irrigation did not reduce Ct values (Figure 2). These results were also observed in a “Ray Ruby” grapefruit orchard on Kuharske citrange (C. sinensis × P. trifoliata) rootstock grown in the same region and soil type in which three planting densities (300, 440, and 975 trees per ha) and two types of CRFs were evaluated [17]. According to these authors, there was no effect of nutritional treatments alone or combination with planting density on the Ct value of the CLas DNA. Conversely, Moreira et al. [16] found that high-density planting resulted in a lower incidence of HLB in sweet oranges, indicating that higher planting densities (714 trees per ha) can dilute psyllid populations per tree compared to low-density plantings (220 trees per ha). Stuchi et al. [42] evaluating citrus tree densities from 714 to 1250 trees per ha, also reported that the HLB progress rates and cumulative HLB incidence were lower at the higher tree densities. These results demonstrate the need
for more evaluations of HLB incidence at high tree densities in long-term studies to identify disease management practices.

Figure 2. Candidatus Liberibacter asiaticus (CLas) DNA in “Ray Ruby” grapefruit (RR) trees expressed in terms of normalized cycle threshold (Ct) values from 2014 to 2018 (a–e). Trees were considered positive when Ct values were ≤32 (indicated by the dashed horizontal line). Treatments: RR/SO_STD_dry_MS = Sour Orange (SO) rootstock + standard spacing (STD, 358 trees per ha) + dry granular fertilizer + microsprinkler (MS), RR/SO_HDS_fert_DD = SO rootstock + high density staggered (HDS, 953 trees per ha) + fertigation (fert) + drip (DD), RR/897_HDS_fert_MS = US-897 rootstock + HDS + fertigation + microsprinkler, RR/897_HDS_fert_DD = US-897 rootstock + HDS + fertigation + drip, and RR/SO_HDS_fert_MS = SO rootstock + HDS + fertigation + microsprinkler. Mean ± standard error followed by different letters within the same year are significantly different at \( p \leq 0.05 \) by Tukey’s test (\( n = 5 \)). NS = Not significant.

3.2. Tree Size

Trunk diameter and canopy volume increased over time as expected (Figure 3a–h) and were higher on RR/SO_STD_dry_MS compared to other treatments. From 2016 to 2017, these parameters were consistently smaller under RR/897_HDS_fert_MS compared to other treatments. Conversely, similar behavior was observed for high-density planting, indicating smaller values for both trunk diameter and canopy volume compared to control (RR/SO_STD_dry_MS) (Figure 3c,d,g,h). These results were expected, since trees planted at lower densities likely receive more solar radiation, water, and nutrients and thus, avoid the level of competition associated with high-density plantings. Closer planting spacings can lead to competition for those resources resulting in lower canopy volume and trunk diameter in grapefruit trees affected by HLB [17].

Previous studies have also demonstrated that trunk diameter and canopy volume decreased at higher planting densities. Wheaton et al. [43] reported that canopy volume of citrus scion cultivars (“Hamlin” and “Valencia” oranges, “Murcott” mandarin, and “Redblush” grapefruit) on 15 different rootstocks varied among different planting densities and found greater canopy volume at lower densities relative to higher densities. Huang [44] similarly found larger canopy volume of “Ponkan” tangerine at lower densities. A study conducted by Zaman and Schumann [45] measuring canopy volume of “Valencia” sweet orange manually and using an ultrasonic device indicated greater canopy volume at lower planting densities. Kumar et al. [46] observed that trunk diameter and canopy volume increased with decrease in plant densities in three almond (Prunus dulcis) variety (“Makhdooom”, “Waris”, and “Shalimar”). A gradual decrease in all canopy parameters with an increase in planting density in six-year-old “Kinnow” mandarin growing on Rough lemon (Citrus jambhiri) rootstock was also demonstrated [15].
The similarity of trunk diameter and canopy volume in high-density planting is likely due to HLB infection, making it difficult for a precise evaluation of irrigation and fertilization effects on these growth parameters. A similar effect was observed in a 27-year old commercial grove planted with “Flame” red grapefruit on Swingle citrumelo (X. Citrocinus spp.) rootstock where canopy growth was severely restricted by HLB infection and did not respond to treatments [47].

To date there is no cure for HLB. Symptom development in HLB-affected trees is related to degeneration of vegetative tissue due to the massive blockage caused by the presence of CLAs in the sieve elements of the phloem [48]. Phloem blockage limits transport of nutrients from leaves to roots and eventually induces dieback of the tree, limiting the availability of nutrients absorbed by the roots to the rest of the plant [38,39]. HLB also causes loss of fibrous roots [36,49]. Other factors that can limit canopy growth include disease severity, which is influenced by several factors, such as tree age, number of infections, and time of infection [50]. Under these conditions, tree growth parameters (e.g., canopy growth, trunk diameter, height, biomass etc.) are highly affected by the disease.

3.3. Foliar Nutrient Concentration

Foliar concentrations of P, K, Cu, Fe, Mn, and Zn varied through time (Tables 1 and 2). Foliar N and P concentrations were not affected by treatments, while treatment effects of Ca and Mg concentrations were detected in 2014. Despite treatment differences, foliar nutrient concentrations were within a narrow range. There was a significant reduction (Ca, p = 0.0040; Mg, p = 0.0003) in foliar concentrations of Ca (27.16 g kg\(^{-1}\)) in RR/897 HDS fert DD and Mg (3.04 g kg\(^{-1}\)) in RR/897 HDS fert MS compared to other treatments. There were also significant reductions in foliar K concentration in 2015 (16.47 g kg\(^{-1}\); p = 0.0225) and 2016 (8.38 g kg\(^{-1}\); p = 0.0004) as well as S concentration in 2016 (2.46 g kg\(^{-1}\)) under RR/897 HDS fert MS (Table 1).
Table 1. Foliar macronutrient concentration of “Ray Ruby” grapefruit trees (RR) as a function of treatments applied during the experimental period (2014–2017).

| Treatment                     | 2014   | 2015   | 2016   | 2017   | 2014   | 2015   | 2016   | 2017   |
|-------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| N g kg⁻¹                      |        |        |        |        |        |        |        |        |
| RR/SO_STD_dry_MS             | 27.27  | 26.72  | 23.55  | 27.13  | 1.52   | 1.40   | 1.90   | 1.82   |
| RR/SO_HDS_fert_DD            | 29.14  | 26.41  | 23.18  | 25.56  | 1.50   | 1.33   | 1.96   | 1.90   |
| RR/897_HDS_fert_MS           | 30.18  | 25.96  | 22.12  | 26.04  | 1.56   | 1.42   | 1.92   | 1.94   |
| RR/897_HDS_fert_DD           | 28.76  | 27.72  | 23.38  | 25.22  | 1.52   | 1.37   | 2.18   | 1.80   |
| RR/SO_HDS_fert_MS            | 28.90  | 29.02  | 22.74  | 25.54  | 1.56   | 1.38   | 1.92   | 1.86   |
| **Optimal range** 1          |        |        |        |        |        |        |        |        |
| **p-value**                  | 0.0766 | 0.1211 | 0.1085 | 0.2223 | 0.3961 | 0.2005 | 0.5107 |        |
| **CV (%)**                   | 5.12   | 6.96   | 3.79   | 5.15   | 7.43   | 10.22  | 7.31   |        |
| Ca g kg⁻¹                    |        |        |        |        |        |        |        |        |
| RR/SO_STD_dry_MS             | 28.42  | 33.47  | 36.35  | 34.90  | 3.55   | 2.69   | 3.13   | 2.98   |
| RR/SO_HDS_fert_DD            | 30.94  | 32.71  | 38.08  | 34.34  | 3.96   | 2.74   | 2.88   | 2.98   |
| RR/897_HDS_fert_MS           | 30.46  | 36.30  | 32.02  | 32.62  | 4.16   | 2.74   | 3.22   | 2.84   |
| RR/897_HDS_fert_DD           | 27.16  | 36.47  | 37.28  | 38.08  | 3.80   | 2.82   | 2.90   |        |
| **Optimal range** 1          |        |        |        |        |        |        |        |        |
| **p-value**                  | 0.0040 | 0.1935 | 0.7953 | 0.3244 | 0.0003 | 0.1176 | 0.4663 |        |
| **CV (%)**                   | 5.76   | 8.38   | 11.03  | 6.30   | 8.76   | 8.78   | 9.40   |        |
| Mg g kg⁻¹                    |        |        |        |        |        |        |        |        |
| RR/SO_STD_dry_MS             | 19.30  | 18.79  | 12.12  | 14.40  | 2.95   | 3.57   | 3.32   | 3.25   |
| RR/SO_HDS_fert_DD            | 18.80  | 19.29  | 13.10  | 15.16  | 2.72   | 3.24   | 3.02   | 3.12   |
| RR/897_HDS_fert_MS           | 18.74  | 16.47  | 8.38   | 13.90  | 2.72   | 3.40   | 2.46   | 2.84   |
| RR/897_HDS_fert_DD           | 17.86  | 21.04  | 15.10  | 15.18  | 2.84   | 3.51   | 3.18   | 3.10   |
| RR/SO_HDS_fert_MS            | 18.52  | 19.64  | 11.52  | 14.18  | 2.92   | 3.48   | 2.82   | 2.82   |
| **Optimal range** 1          |        |        |        |        |        |        |        |        |
| **p-value**                  | 0.7895 | 0.0225 | 0.0004 | 0.1567 | 0.8453 | 0.3794 | 0.0011 | 0.0418 |
| **CV (%)**                   | 9.63   | 10.27  | 15.79  | 6.53   | 14.38  | 7.96   | 9.68   | 7.92   |

1 Optimal nutrient concentration as reported by Morgan et al. [28]. Treatments: RR/SO_STD_dry_MS = Sour Orange (SO) rootstock + standard spacing (STD, 358 trees per ha) + dry granular fertilizer + microsprinkler (MS), RR/SO_HDS_fert_DD = SO rootstock + high density staggered (HDS, 953 trees per ha) + fertigation (fert) + drip (DD), RR/897_HDS_fert_MS = US-897 rootstock + HDS + fertigation + microsprinkler, RR/897_HDS_fert_DD = US-897 rootstock + HDS + fertigation + drip, and RR/SO_HDS_fert_MS = SO rootstock + HDS + fertigation + microsprinkler. CV = coefficient of variation. Means followed by different lowercase letters are significantly different at p ≤ 0.05 by Tukey’s test (n = 5). NS = Not significant. * and ** = Significant at p ≤ 0.05 and p ≤ 0.01, respectively.
Table 2. Foliar micronutrient concentration of “Ray Ruby” grapefruit (RR) trees as a function of treatments applied during the experimental period (2014–2017).

| Treatment                          | 2014 | 2015 | 2016 | 2017 |
|------------------------------------|------|------|------|------|
|                                    | B (mg kg\(^{-1}\)) | Cu (mg kg\(^{-1}\)) | Fe (mg kg\(^{-1}\)) | Mn (mg kg\(^{-1}\)) | Zn (mg kg\(^{-1}\)) |
| RR/SO_STD_dry_MS                   | 71.62 a | 298.58 a | 73.92 a | 137.50 a | 72.65 a |
| RR/SO_HDS_fert_DD                 | 71.61 a | 49.60 a | 82.91 ab | 35.29 a | 18.54 a |
| RR/897_HDS_fert_MS                | 51.69 bc | 200.46 a | 94.42 a | 136.68 a | 70.18 a |
| RR/897_HDS_fert_DD                | 53.18 a | 228.64 a | 111.40 a | 110.19 a | 25.36 a |
| RR/SO_HDS_fert_MS                 | 48.96 bc | 231.68 a | 82.47 a | 26.97 a | 18.54 a |
| RR/SO_HDS_fert_MS                 | 70.42 ab | 260.04 a | 94.56 a | 32.86 a | 25.51 a |
| RR/SO_HDS_fert_MS                 | 57.64 ab | 233.98 a | 97.25 a | 33.73 a | 22.93 a |

Optimal range 1

| Treatment                          | 2014 | 2015 | 2016 | 2017 |
|------------------------------------|------|------|------|------|
|                                    | Cu (mg kg\(^{-1}\)) | Fe (mg kg\(^{-1}\)) | Mn (mg kg\(^{-1}\)) | Zn (mg kg\(^{-1}\)) |
| RR/SO_STD_dry_MS                   | 60–120 | 5–16 | 25–100 | 25–100 |
| RR/SO_HDS_fert_DD                 | 160–120 | 5–16 | 160–120 | 25–100 |
| RR/897_HDS_fert_MS                | 125–100 | 5–16 | 160–120 | 25–100 |
| RR/SO_HDS_fert_MS                 | 125–100 | 5–16 | 160–120 | 25–100 |

Optimal nutrient concentration as reported by Morgan et al. [28]. Treatments: RR/SO_STD_dry_MS = Sour Orange (SO) rootstock + standard spacing (STD, 358 trees per ha) + dry granular fertilizer + microsprinkler (MS), RR/SO_HDS_fert_DD = SO rootstock + high density staggered (HDS, 953 trees per ha) + fertigation (fert) + drip (DD), RR/897_HDS_fert_MS = US-897 rootstock + HDS + fertigation + microsprinkler, CV = coefficient of variation. Means followed by different lowercase letters are significantly different at \( p \leq 0.05 \) by Tukey’s test (\( n = 5 \)). NS = Not significant. * and ** = Significant at \( p \leq 0.05 \) and \( p \leq 0.01 \), respectively.

No significant differences were found between treatments in foliar Cu, Mn, and Zn concentrations. During the study, foliar B concentrations were higher in the standard treatment (lowest tree density and soil-applied CRF) when compared to the fertigation and high-density plantings. In 2015, foliar Fe
concentration was the lowest (71.34 mg kg\(^{-1}\)) in RR/SO_HDS_fert_DD and the highest (89.49 mg kg\(^{-1}\)) in RR/SO_HDS_fert_MS, indicating the increase in foliar Fe concentration was probably from the microsprinkler irrigation system used on those treatments (Table 2).

Foliar nutrient concentrations were compared to optimum values defined by Morgan et al. [28]. Except for Cu during the entire study and Mn in 2014, foliar nutrient concentrations were within or higher than optimal ranges (Tables 1 and 2), indicating that all treatments were effective in meeting tree nutrient demand. Kadyampakeni et al. [3] similarly reported that sweet orange foliar N, P, and K concentrations were optimal or higher under drip and microsprinkler fertigation on a coastal Flatwoods soil, while a study on a well-drained soil in Central Florida showed that CRF and microsprinkler fertigation resulted in similar foliar N concentrations in young sweet orange orchards [2].

Foliar Cu concentration was consistently high (>16 mg kg\(^{-1}\)) due to the frequent application of copper-based fungicides to control citrus canker. The increase in foliar Cu concentration may have caused the reduction of Zn levels. This was more evident under RR/897_HDS_fert_MS during the last three years of evaluation (2015–2017) with foliar Zn concentration below the optimal range (>25 mg kg\(^{-1}\)) recommended by Morgan et al. [28].

Antagonisms between Cu and Zn for their absorption at the surface of leaves has been well-documented in the literature [51]. Additionally, in 2014, Mn was above sufficiency range for plant tissue (>100 mg kg\(^{-1}\)). Factors influencing increases in foliar Mn levels are not well understood.

Although the concentration of some nutrients was above or slightly below the optimal range, no nutritional toxicity and deficiency symptoms were observed during the study. According to Acosta [47], concentrations greater than 100 mg kg\(^{-1}\) for B, Mn, and Zn in leaves can be tolerated by grapefruit trees affected by HLB.

Results from this study showed the combinations of rootstock, high planting density, fertilization and water management using ACPS/OHS did not affect grapefruit foliar nutrient concentrations when compared to the standard treatment (RR/SO_STD_dry_MS). A recent study with HLB-affected grapefruit at three planting densities, two CRF blends applied in the soil, and foliar micronutrient application showed that supplemental nutrient application does not enhance fruit yield in grapefruit trees affected by HLB [17]. However, that study did find that high-density planting (975 trees per ha) was a viable option to manage HLB. Tree density has become a critical consideration in new citrus plantings due to the potential for increasing fruit yield in young orchards [16].

There is limited information on irrigation, fertilizer application, citrus rootstocks, and tree density combined on grapefruit nutrition in ACPS/OHS [12]. Additional research is needed to determine optimal fertilization rates for grapefruit high-density plantings under HLB-endemic conditions. However, few studies have been conducted exploring the effects of nutrient management via soil application and fertigation on grapefruit.

3.4. Number of Fruit, Fruit Size, and Fruit Yield

Fruit number varied substantially among treatments and years, ranging from 16 to 26 fruit in 2015/16 and from 53 to 69 in 2016/17 (Figure 4a,b). For these two years, there was no treatment effect on fruit number (\(p > 0.05\)). In 2017/18, treatment differences were observed (\(p = 0.0256\)) as trees under RR/897_HDS_fert_DD had the greatest number of fruit (Figure 4c).
Our results showed that yields were similar for both rootstocks (Sour Orange and US-897) planted at high density staggered (HDS, 953 trees per ha) + fertigation (fert) + microsprinkler, RR/US-897_HDS_fert_MS = US-897 rootstock + HDS + fertigation + microsprinkler, RR/897_HDS_fert_DD = US-897 rootstock + HDS + fertigation + drip, and RR/SO_HDS_fert_MS = SO rootstock + HDS + fertigation + microsprinkler. Mean ± standard error followed by different letters within the same year are significantly different at p ≤ 0.05 by Tukey’s test (n = 5). NS = Not significant.

A significant amount of small-sized fruit (<100 mm) were observed each year regardless of treatment (Figure 4a–c), which was expected in 2015/16 since trees reached bearing age. These results agree with Ferrarezi et al. [29]. In 2017/18, fruit yield was compromised by Hurricane Irma, which caused 50–70% fruit drop (visual observation). While the fruit number increased in 2016/17 compared to other years, on average 65% of fruit were small (<100 mm), probably due to high HLB incidence. Conversely, fruit produced under RR/897_HDS_fert_DD (Ct value ≥ 32, Figure 2c) had the highest amount of adequate size compared to other treatments (79%). Previous research also reported that fruit number in the small class (<60–63 mm) was significantly greater for HLB-affected trees compared to healthy trees [52], indicating that the percentage of adequate size fruit per tree is smaller on HLB-affected trees compared to healthy trees. For HLB-affected “Valencia” sweet orange, fruit harvested from severely symptomatic trees was significantly smaller and lighter than low and moderately symptomatic trees [41]. Those authors also reported that with the arrival of HLB, a decrease in fruit size has been observed throughout Florida in addition to an increase in pre-harvest fruit drop, suggesting these two problems are correlated.

Fruit yield varied by year (2015/16 = 3824–9272 kg ha⁻¹, 2016/17 = 7310–25,003 kg ha⁻¹, and 2017/18 = 3845–11,192 kg ha⁻¹) depending on treatment (Figure 4d,e). Low yields observed for all treatments during 2015/16 and 2017/18 harvest seasons were related to grapefruit bearing age and hurricane damages in 2017, respectively. Thus, it was only possible to notice large differences between treatments in the 2016/17 harvest season (p = 0.0017), mainly for planting density and fertilizer application. Our results showed that yields were similar for both rootstocks (Sour Orange and US-897) planted at high density using fertigation by drip or microsprinkler and were 67% (average between treatments) higher than the standard treatment (RR/SO_STD_dry_MS) (Figure 4e). These results indicate that...
citrus growers can use any of these irrigation systems to optimize yield, which is important because it indicates that ACPS/OHS results in greater productivity in comparison to the standard treatment (RR/SO_STD_dry_MS). A study conducted in Florida also indicated that OHS system improves growth and yield when drip-irrigated trees (“Hamlin” and “Valencia” oranges) are compared with small-area and large-area microsprinkler-irrigated trees [12]. Kadyampakeni et al. [8] showed that daily water use was similar or greater using the drip irrigation compared to the conventional grower practices. The authors suggest that frequent irrigation (pulsed frequently with drip or irrigated once a day with microsprinklers) increases water availability and promotes root length and density in the irrigated zone, which can in turn increase water uptake.

Fruit yield was greater under ACPS/OHS compared to standard treatment (RR/SO_STD_dry_MS) (Figure 4). Thus, our study showed high-density planting in OHS is a promising way to increase fruit yield per unit area. Schumann et al. [10] observed that planting density was identified as the most significant contributor to higher early yields of “Hamlin” orange. The authors also noticed that the integration of OHS, high-density planting, and superior rootstock combinations can produce early high fruit yields. However, information for citrus production under ACPS/OHS conditions, particularly with HLB-affected grapefruit remains scarce. Conversely, studies conducted prior to the HLB era in Florida have shown that grapefruit trees bear more fruit even when subjected to rigid tree size control at high-density planting [43]. Previous studies have also demonstrated that high-density plantings result in greater production per area [53,54] especially under HLB-endemic conditions [9,16].

3.5. Fruit Quality

Fruit quality parameters in high-density grapefruit production in OHS were measured in 2016/17 and 2017/18 (Figure 5). Densely planted orchards increase yield per area (Figure 4e), but also increase the shading and competition for solar radiation, which may reduce photosynthesis and the supply of sugars and nutrients to fruit, reducing fruit yield and quality [55]. Water competition in the tree root zone may also affect fruit quality at high-density plantings. Hutton and Loveys [56] suggested that reduction in water input in mature “Bellamy” nucellar navel orange trees on P. trifoliata rootstock resulted in significant reductions in juice volume and an increase in acidity in the experimental period without changing SSC. However, our study demonstrated that SSC, acidity, and SSC-to-titratable acidity ratio were not affected by planting density. Conversely, Phuyal et al. [17] showed that “Ray Ruby” grapefruit on Kuharske citrange planted high-density (975 trees per ha) in Flatwoods soil tended to accumulate more soluble solids than under low-density planting (300 trees per ha).

Fruit quality parameters were not influenced by rootstock, fertilization methods, and irrigation systems (Figure 5). Fruit quality parameters of “Ray Ruby” grapefruit grown on seven rootstocks (including US-897 and Sour Orange) were also evaluated in a commercial orchard trial in the same region [57]. The authors observed that US-942 and US-897 rootstocks produced fruit with quality characteristics that equaled or exceeded Sour Orange and Swingle, the two most common rootstocks used in the Indian River District.
Fruit harvested in 2016/17 showed higher SSC ranging from 9.3% to 9.6% compared to 7.32% to 7.68% in the 2017/18 harvest season (Figure 5a,b). Grapefruit acidity ranged from 0.83% to 0.92% citric acid in 2016/17 (Figure 5c). In 2017/18 harvest season, we found titratable acidity values ranging from 0.87% to 0.98% citric acid (Figure 5d). Additionally, the SSC-to-titratable acidity ratio was highest in 2016/17 (values ranging from 10.35 to 11.28) compared to obtained values (from 7.82 to 8.43) in 2017/18 harvest season (Figure 5e,f). In the USA, a minimum and maximum SSC-to-titratable acidity ratio ranging from 7:1 to 14:1 is typically desired for grapefruit [58]. Thus, despite high HLB incidence and hurricane damage in 2017, fruit quality parameters remained within industry adequate standards. Similar results were reported by McCollum and Bowman [57] for “Ray Ruby” grapefruit grown on Sour Orange (SSC = 8.48%, acidity = 1.12% citric acid, and SSC-to-titratable acidity ratio = 7.56; average values) and US-897 (SSC = 9.44%, acidity = 1.20% citric acid, and SSC-to-titratable acidity ratio = 7.85; average values). A recent study by Phuyal et al. [17] also showed similar results for fruit quality parameters (SSC = 7.59%–8.55%, acidity = 1.00%–1.18% citric acid, and SSC-to-titratable acidity ratio = 7.85–8.43).
ratio = 7.26–8.08) due to CRF application and planting density in 2017/18 and 2018/19 harvest seasons. Both studies were conducted in HLB-affected grapefruit.

4. Conclusions

All trees were positive for CLAs five years after planting. Trees planted at standard density developed a larger trunk diameter and canopy volume. A hurricane in 2017 caused major fruit drop, resulting in smaller number of fruit and decreased fruit size in the subsequent season. Nevertheless, these factors did not affect fruit quality. HDS planting resulted in higher fruit yield, irrespective of rootstock (“Sour Orange” and “US-897”) and irrigation system (drip or microsprinkler), representing an important advance to grapefruit production systems.

Our results collected in this large-scale field trial demonstrate that high-density grapefruit production in OHS can be used by citrus growers who aim to make the best water and nutrient management decisions in HLB-affected orchards. It is likely that improved approaches to fertilization and irrigation rates will require adjustments to OHS based on HLB incidence. Therefore, further long-term research is needed to evaluate which treatment combination will provide the greatest economic benefits and is practical to implement by growers. However, labor costs and effects on plant growth over time still need to be evaluated for commercial recommendations.

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References

1. USDA. Florida citrus statistics 2018–2019. Florida Department of Agriculture and Consumer Services. National Agricultural Statistics Service. Available online: https://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/Commercial_Citrus_Inventory/Commercial_Citrus_Inventory_Prelim/ccipr19.pdf (accessed on 4 May 2020).
2. Morgan, K.T.; Wheaton, T.A.; William, S.C.; Parsons, L.R. Response of young and maturing citrus trees grown on a sandy soil to irrigation scheduling, nitrogen fertilizer rate, and nitrogen application method. HortScience 2009, 44, 145–150. [CrossRef]
3. Kadyampakeni, D.M.; Morgan, K.T.; Schumann, A.W. Biomass, nutrient accumulation and tree size relationships for drip- and microsprinkler-irrigated orange trees. J. Plant Nutr. 2016, 39, 589–599. [CrossRef]
4. Ferrarezi, R.S.; Rodriguez, K.; Sharp, D. How historical trends in Florida all-citrus production correlate with devastating hurricane and freeze events. Weather 2020, 75, 77–83. [CrossRef]
5. Dala-Paula, B.M.; Plotto, A.; Bai, J.; Manthey, J.A.; Baldwin, E.A.; Ferrarezi, R.S.; Gloria, M.B.A. Effect of Huanglongbing or greening disease on orange juice quality, a review. Front. Plant Sci. 2019, 9, 1–19. [CrossRef] [PubMed]
6. Kadyampakeni, D.M.; Morgan, K.T.; Schumann, A.W.; Nkedi-Kizza, P. Effect of irrigation pattern and timing on root density of young citrus trees infected with Huanglongbing disease. HortTechnology 2014, 24, 209–221. [CrossRef]
29. Ferrarezi, R.S.; Qureshi, J.A.; Wright, A.L.; Ritenour, M.A.; Macan, N.P.F. Citrus production under screen as a strategy to protect grapefruit trees from Huanglongbing disease. *Front. Plant Sci.* 2019, 10, 1–15. [CrossRef]

30. Wardowski, W.; Whigham, J.; Grierson, W.; Soule, J. Quality Tests for Florida Citrus. 1995. Available online: [http://irrec.ifas.ufl.edu/postharvest/pdfs/QualityTests_for_FL_Citrus_SP_99.pdf](http://irrec.ifas.ufl.edu/postharvest/pdfs/QualityTests_for_FL_Citrus_SP_99.pdf) (accessed on 7 April 2020).

31. Gottwald, T.R.; Graham, J.H.; Irey, M.S.; McColllum, T.G.; Wood, B.W. Inconsequential effects of nutritional treatments on Huanglongbing control, fruit quality, bacterial titer and disease progress. *Crop. Prot.* 2012, 36, 73–82. [CrossRef]

32. SAS Institute Inc. *Base SAS®9.4 Procedures Guide*; SAS Institute Inc.: Cary, NC, USA, 2013.

33. Shin, K.; Van Bruggen, A.H.C. Bradyrhizobium isolated from Huanglongbing (HLB) affected citrus trees reacts positively with primers for *Candidatus Liberibacter asiaticus*. *Eur. J. Plant Pathol.* 2018, 151, 291–306. [CrossRef]

34. Morgan, K.T.; Rouse, R.E.; Ebel, R.C. Foliar applications of essential nutrients on growth and yield of ‘Valencia’ sweet orange infected with Huanglongbing. *HortScience* 2016, 51, 1482–1493. [CrossRef]

35. Bove, J.M. Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. *J. Plant Pathol.* 2006, 88, 7–37.

36. Graham, J.H.; Johnson, E.G.; Gottwald, T.R.; Irey, M.S. Presymptomatic fibrous root decline in citrus trees caused by Huanglongbing and potential interaction with *Phytophthora* spp. *Plant Dis.* 2013, 97, 1195–1199. [CrossRef] [PubMed]

37. Etxeberria, E.; Gonzalez, P.; Achor, D.; Albrigo, G. Anatomical distribution of abnormally high levels of starch in HLB-affected Valencia orange trees. *Physiol. Mol. Plant P.* 2009, 74, 76–83. [CrossRef]

38. Achor, D.; Etxeberria, E.; Wang, N.; Folimonova, S.; Chung, K.; Albrigo, L. Sequence of anatomical symptom observations in citrus affected with Huanglongbing disease. *Plant Pathol.* J. 2010, 9, 56–64. [CrossRef]

39. Folimonova, S.Y.; Achor, D.S. Early events of citrus greening (Huanglongbing) disease development at the ultrastructural level. *Plant Pathol. J.* 2010, 100, 949–958. [CrossRef]

40. Handique, U.; Ebel, R.; Morgan, K.T. Influence of soil-applied fertilizer on greening development in new growth flushes of sweet orange. *Proc. Fla. State. Hort. Soc.* 2012, 125, 36–39.

41. Vashisth, T.; Tang, L. Fruit Drop and HLB. *Citrus Industry*. Available online: [http://citrusindustry.net/2018/09/12/fruit-drop-and-hlb/](http://citrusindustry.net/2018/09/12/fruit-drop-and-hlb/) (accessed on 27 April 2020).

42. Stuchi, E.S.; Girardi, E.A.; Basssanezi, R.B.; Laranjeira, F.F. Yield and Huanglongbing progress at four tree spacings of sweet orange. In Proceedings of the International Citrus Congress, Londrina, Brazil, 18–23 September 2016; pp. 56–5299.

43. Wheaton, T.A.; Castle, W.S.; Whitney, J.D.; Tucker, D.P.H. Performance of citrus scion cultivars and rootstock in a high-density planting. *Hort. Sci.* 1991, 26, 837–840. [CrossRef]

44. Huang, R.F. Study on the effect of planting density on the growth and production of Ponkan mandarin. *South China Fruit* 1997, 26, 5–21.

45. Zaman, Q.U.; Schumann, A.W. Performance of an ultrasonic tree volume measurement system in commercial citrus groves. *Precis. Agric.* 2005, 6, 467–480. [CrossRef]

46. Kumar, D.; Ahmad, N.; Verma, M.K. Studies on high density planting in almond in Kashmir valley. *Indian J. Hort.* 2012, 69, 328–332.

47. Acosta, D.F.R. Mitigation of Huanglongbing Effects on Grapefruit Trees Using Enhanced Nutritional Programs. Master’s Thesis, University of Florida, Gainesville, FL, USA, 2016.

48. Li, J.; Li, L.; Pang, Z.; Kolbasov, V.G.; Ehsani, R.; Carter, E.W.; Wang, N. Developing citrus Huanglongbing (HLB) management strategies based on the severity of symptoms in HLB-endemic citrus-producing regions. *Physopathology* 2019, 109, 582–592. [CrossRef]

49. Gonzalez, P.; Reyes-De-Corcuera, J.; Etxeberria, E. Characterization of leaf starch from HLB-affected and unaffected-girdled citrus trees. *Physiol. Mol. Plant Pathol.* 2012, 79, 71–78. [CrossRef]

50. Gottwald, T.R. Epidemiological understanding of citrus Huanglongbing. *Annu. Rev. Phytopathol.* 2010, 48, 119–139. [CrossRef]

51. Mann, M.S.; Takkar, P.N. Antagonism of micronutrient cations on sweet orange leaves. *Sci. Hortic.* 1983, 20, 259–265. [CrossRef]

52. Spann, T.M.; Danyluk, M.D. Effects of HLB Infection on Sweet Orange Fruit Size and Quality. Available online: [https://crec.ifas.ufl.edu/extension/trade_journals/2010/2010_sept_effects_hlb.pdf](https://crec.ifas.ufl.edu/extension/trade_journals/2010/2010_sept_effects_hlb.pdf) (accessed on 27 April 2020).
53. Hutton, R.J.; Broadbent, P.; Bevington, K. Viroid dwarfing for high density citrus planting. *Hortic. Rev.* **2000**, *24*, 277–317.

54. Vidalakis, G.; Pagliaccia, D.; Bash, J.A.; Afunian, M.; Semancik, J.S. Citrus dwarfing viroid: Effects on tree size and scion performance specific to Poncirus trifoliata rootstock for high-density planting. *Ann. Appl. Biol.* **2011**, *158*, 204–217. [CrossRef]

55. Weibel, F.P.; Alföldi, T. Improving the quality and shelf life of fruit from organic production systems. In *Handbook of Organic Food Safety and Quality*, 1st ed.; Cooper, J., Leifert, C., Niggli, U., Eds.; Woodhead Publishing: Sawston, Cambridge, UK, 2007; pp. 330–352.

56. Hutton, R.J.; Loveys, B.R. A partial root zone drying irrigation strategy for citrus-effects on water use efficiency and fruit characteristics. *Agric. Water Manag.* **2011**, *98*, 1485–1496. [CrossRef]

57. McCollum, G.; Bowman, K.D. Rootstock effects on fruit quality among ‘Ray Ruby’ grapefruit trees grown in the Indian River District of Florida. *HortScience* **2017**, *52*, 541–546. [CrossRef]

58. USDA. United States Standards for Grades of Grapefruit Juice. 2012. Available online: https://www.ams.usda.gov/sites/default/files/media/Canned_Grapefruit_Juice_Standard%5B1%5D.pdf (accessed on 1 May 2020).

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