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# Performance of Two Citrus Species Grafted to Different Rootstocks in the Presence of Huanglongbing Disease in Puerto Rico

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**Abstract:** Since Huanglongbing (HLB) disease was detected in 2009 in Puerto Rico, a steady drop in citrus production has been experienced, forcing farmers to abandon their land or switch to other crops. Between 2015 and 2016, we used grafted trees from two experimental orchards (Tahiti lime and Nova mandarin), each on five rootstocks, to collect soil and plant tissue samples from each scion-rootstock combination to determine soil fertility, tissue nutrient content, and yield. The tree growth parameters (height, diameter, and canopy volume) and efficiency of the two orchards were also measured. These orchards, growing in Coto series (Typic Hapludox), were planted in 2009 and reported as heavily infested with HLB by 2011. Our results showed that soil and tissue samples from the Tahiti lime orchard exhibited benefits for tree growth parameters when grafted on Carrizo and Cleopatra rootstocks. Lower tree mortality (13%) was observed for Tahiti lime grafted on Carrizo, HRS 812, Carrizo and Rough lemon rootstocks, while 25% of the Nova mandarin trees perished on the same rootstocks. Yield was higher for Tahiti lime grafted on Swingle rootstock (35.6 fruit $m^{-3}$) as compared to the other rootstocks. In general, HLB appears to have caused poor development and low production in the Nova mandarin orchard.

**Keywords:** Huanglongbing; Tahiti lime; Nova mandarin; rootstocks; scions

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## 1. Introduction

Success in citrus production depends on the selection of suitable high-quality scion-rootstock combinations, which at the same time are adaptable to a wide range of environmental conditions and tolerant to various pests and diseases. No condition is more serious in citrus than Huanglongbing (HLB) disease, a lethal vector-transmitted disease. The causal agent of the disease is the bacterium *Candidatus Liberibacter asiaticus* (*CLas*), vectored by the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama [1]. Citrus trees infected with HLB present asymmetric foliar chlorosis, acidic fruits, a shortened life cycle, branch dieback, and eventually tree death [2,3].

In Puerto Rico, HLB-ACP was first detected in 2001 in the Agricultural Experiment Substation (AES) of Isabela; the presence of the bacteria *CLas* was first identified there in 2009 [2,4], in commercial orchards of sweet orange [*Citrus sinensis* (L.) Osbeck] and lemon (*Citrus latifolia*), and in lemon orchards at AES of Juana Díaz [5–7]. From 2012 to 2015, the island experienced a 39% reduction (i.e., 2556 tons to 1557 tons) in citrus production [8]. In 2012 the citrus industry was ranked second among fruit commodities in PR, with over 7000 ha planted on 2800 farms (~700 producers), most of them located in the mountainous region of the island. Between 2013 and 2014, the citrus industry ranked third, with a net value of $6 million as reported by the Department of Agriculture of Puerto Rico (DAPR) [9,10]. Over 68 million fruits were produced during that year. However, since HLB and *CLas* were identified, the disease has spread throughout the entire island and the citrus industry has experienced a steady drop in production, forcing farmers to abandon their land and/or switch to alternate crops, such as coffee or plantains, usually facing high economic losses [11]. In addition, this decline in citrus production appears to be more severe in orange and mandarin orchards than in lime or lemon orchards [11].

The HLB problem may be compounded by the higher susceptibility to certain fungal (i.e., *Phythophtora* spp.) diseases that have been found in varietals grafted to Cleopatra mandarin rootstock (*Citrus reshni* Hort. Ex Tan) [12], which until recently (up to 2013) was the predominant rootstock used by farmers in PR [13]. After 2013, three citrus rootstocks [i.e., Swingle citrumelo, Carrizo citrange, and HRS 812 (also known as US 812)] were highly recommended to farmers by Román-Pérez et al. [14] after being evaluated for several years at different locations and with diverse scion-rootstock combinations. These three rootstocks had shown great potential in mainland USA, although little was known of their potential for commercial production in PR. A study driven by Román-Pérez and González-Vélez [13] found that the three rootstocks had similar horticultural responses to scions on Cleopatra rootstock. However, through the years, Cleopatra showed more susceptibility to *P. citrophthora* with increasing tree mortality, and Swingle citrumelo and Carrizo rootstocks have shown some resistance to Citrus Tristeza virus (CTV) and the scion-HRS 812 rootstock exhibited 100% survival with CTV [13].

Bowman and Rouse [15] found that HRS 812 rootstock was highly productive in Florida with high-quality fruits, and exhibited tolerance to CTV. Albrecht and Bowman [16] found in a laboratory trial that Carrizo and HRS-897 (also known as US 897) were tolerant to HLB, while HRS 812 was considered moderately tolerant to HLB compared to Cleopatra mandarin. The transmission of the HLB causal agent CLas was limited to the plant phloem, and was attributed primarily to ACP and, secondarily, to human-mediated transmission by grafting [17].

Spann et al. [18] attributed nutrient deficiencies to HLB, where infected plants had significantly lower values of the macronutrients calcium (Ca), phosphorus (P), and sulfur (S), and the micronutrients manganese (Mn), iron (Fe), and copper (Cu). Studies by Gottwald et al. [19] indicated no effective response to insecticide application to reduce ACP population, nor to an enhanced nutritional program on two trials conducted with Valencia orange in Florida. In Brazil, even though infected trees were removed and intensive ACP management practices were performed, the HLB disease has spread exponentially, causing significant yield losses [20]. Improved plant nutrition may help to slow down HLB disease progression in a tree, but will not cure them of HLB.

Scientifically-based fertilization data for overcoming damage caused by HLB is scarce. Accurate HLB detection requires DNA tests, since visual symptoms are similar to micronutrient deficiencies and other citrus diseases such as citrus variegated chlorosis, citrus cankers, etc. In terms of nutrition, most of the recommendations provided by local Agricultural Extension Services and the Puerto Rico Department of Agriculture are based on data and recommendations gathered from citrus growers from the mainland and Florida-USA Cooperative Extension Service publications [11]. However, significant differences exist in soil type, topography, and climate between Florida and Puerto Rico, and so the inherent limitations of such recommendations are a serious concern. For that reason, our objective was to evaluate the role of rootstocks on the performance of two HLB-infected mature citrus orchards established at AES in Isabela in overcoming the effect of this disease.

## 2. Materials and Methods

### 2.1. Experimental Area and Description

Two experimental orchards [Tahiti lime and Nova mandarin] were planted in 2009 in the Agricultural Experiment Substation (AES) at Isabela, PR. The AES at Isabela is located in the northwest of the island (18.46 N and 67.05 W) at 120 m above sea level. Both citrus orchards were established in Coto series (*Very fine*, *kaolinitic*, *isohyperthermic*, *Typic Hapludox*) soil [21]. The annual average precipitation is 1639 mm, with May the rainiest month and February the driest month. The maximum average temperature is 29 ± 6 °C.

Tahiti lime (*Citrus latifolia* Tan) scions were grafted to five different citrus rootstocks: Carrizo citrange [*C. sinensis* × *P. trifoliata* (L.) Raf.], Cleopatra mandarin [*Citrus reshni* Hort. Ex Tan], HRS 812 [Sunki mandarin, *C. reticulata* × *P. trifoliata* (L.) Raf.], Rough lemon [*C. jambhiri*], and Swingle citrumelo [*C. paradise* Macf. × *P. trifoliata* (L.) Raf.]. Nova mandarin (*Citrus reticulata*) scions were grafted to five different citrus rootstocks: Carrizo, HRS 812, HRS 896 (also known as US 896) [*Cleopatra mandarin* × *Rubidoux trifoliate orange*], Rough lemon, and Swingle citrumelo.

Both experimental orchards were established as a Randomized Complete Block Design (RCBD) with four replicates. The trees were set at a distance of 4.5 by 5.9 m and each experimental plot had two trees. A supplementary drip irrigation system was installed and used as needed. Both experimental orchards were six years old at the time of our observations, had similar management practices, and showed HLB symptoms by 2011. Presence of HLB was confirmed in 2013 using a polymerase chain reaction (PCR) amplification of bands of 1160 bp corresponding *CLas* bacteria [6,22]. *CLas* titers were not determined. Orchards received 1.8 kg per tree of slow-release fertilizer (15-3-19-3) with micronutrients twice a year. Since the establishment of the two citrus experimental orchards in 2009 up to 2015, the trees were limed once a year [13]. From 2009 to 2010, the citrus trees were fertilized and managed as suggested in the "Conjunto Tecnológico para la producción de Cítricos en Puerto Rico" [23]. To overcome the effects of HLB on tree phloem, the fertilization program was modified since 2011–2016, with slow release fertilizers and supplementary foliar nutritional cocktail, both with micronutrients as recommended it by Rouse [24] in the state of Florida, USA. The supplementary foliar nutritional cocktail with micronutrients consisted of slow release nitrogen (30-0-0), Phosphite® (0-29-26), Recover Rx® (3-18-18) and a biological fungicide (Companion®) were applied in monthly basis. In addition, systemic insecticide Admire Pro® (active ingredient Imidacloprid) was applied bimonthly from April to December 2015 and April–May 2016 to control ACP. Higher dosages (14 oz/75 gals of water) of Imidacloprid were used during peak ACP periods (April and June) and lower dosages (7 oz/75 gals of water) from August to December.

### 2.2. Variables Measured

In late April 2016 composite soil samples were collected from each scion-rootstock combination and replicated by using a 7.62 cm bucket auger, and were analyzed to determine soil fertility. Soil pH was measured in a 1:1 ($v/v$) soil-water mixture [25]. Exchangeable calcium ($Ca^{+2}$), magnesium ($Mg^{2+}$), sodium ($Na^{+}$) and potassium ($K^{+}$) were extracted using 1 mol $L^{-1}$ $NH_4OAc$ [26] and available P by Olsen extract. Organic matter (OM) was determined by loss on ignition. Total sulfur (S) was determined via inductively coupled plasma spectrometry (ICP) (Teledyne Leeman Labs Prodigy Dual, Hudson, NH, USA) after Perchloric acid digestion [27,28]. Nitrate ($NO_3$-N) content (1:1 soil: distilled (DI) water) was determined using a Nitrate-Nitrite Astoria Pacific 2 analyzer (Portland, OR, USA). Also, citrus tissue samples from each scion-rootstock combination and replicate were collected in late April 2016 and analyzed for nitrogen (N), Ca, Mg, P, K, Mn, Fe, Cu, boron (Bo), aluminum (Al), Na, and zinc (Zn) after extraction using Mehlich 3.

Tree variables (i.e., height, diameter, and canopy volume) and fruit yield were measured to determine citrus crop performance. Tree variables in both orchards were measured in May 2016. Tree height and diameter were measured using a telescoping-measuring pole. Tree canopy volume (TCV) was calculating using the Fallahi and Mousavi [29] equation: $0.524 \times \text{tree height (m)} \times \text{tree diameter (m}^2\text{)}$. Tree yield efficiency was calculated using the total average fruit number divided by TCV. Fruit production in the Tahiti lime orchard was measured seven times between April 2015 and May 2016. The Nova mandarin orchard was measured once in February 2016. Fruit production (fruit number and size) was considered for this manuscript as the total average value.

### *2.3. Statistical Analysis*

Analysis of variance (ANOVA) followed by mean separation using Tukey's Honest Significant Difference test at $p < 0.10$ for the RCBD design was used to determine soil and tissue fertility and tree variables and efficiency of the different scion-rootstock combinations. Statistical analysis was undertaken using JMP Version 10 (SAS Institute, Cary, NC, USA).

## 3. Results

### *3.1. Soil Chemical Properties*

In the Tahiti lime orchard, the soil concentrations of Mg, Na, S, and $NO_3$-N for the different scion-rootstock combinations were statistically different. Soil collected from where Carrizo rootstocks grew had higher Mg and Na concentrations than that collected around HRS 812 (Table 1). However, higher concentrations of S and $NO_3$-N were found under HRS 812 and Cleopatra than the other rootstocks. In the Nova mandarin orchard, only OM and Na concentrations in the soil were statistically different. Higher OM content was found in soil collected from where Carrizo (5.05%) rootstocks were grown compared with the other (~4.58%) rootstocks. In addition, the soil collected from where HRS 896 rootstocks were grown had a higher Na (14.8 mg $kg^{-1}$) concentration than Carrizo (10 mg $kg^{-1}$) rootstock soil. However, no statistical differences were found for the other studied variables (Table 1).

### *3.2. Tissue Nutrient and Trace Element Concentrations*

In Tahiti lime tissue samples, K, Mg, N, and Mn concentrations were affected when grafted to five different rootstocks (Table 2). Higher concentrations of K were found in Tahiti lime grafted to Rough lemon and Swingle compared to the other three rootstocks (Table 2). For Mg concentrations, higher significant differences were found between Tahiti lime scions grafted to Cleopatra (0.383 mg $kg^{-1}$) rootstock than those grafted to Swingle (0.275 mg $kg^{-1}$) rootstock. However, for N concentrations, a higher significant difference was found between Tahiti lime scions grafted to Swingle (2.88%) compared to Cleopatra (2.48%). Higher Mn concentrations were found for Tahiti lime scions grafted to Cleopatra (101 mg $kg^{-1}$) rootstock versus those grafted to Carrizo (48.3 mg $kg^{-1}$) (Table 2). No statistical differences were found in Tahiti lime tissue samples for Ca, Na, P, and S concentrations grafted to the five different rootstocks (Table 2).

In Nova mandarin orchards, only Ca, Mg, P, N, and Mn concentrations appeared to be affected when grafted to different rootstocks (Table 2). Higher Ca concentrations were found in Nova mandarin grafted to Carrizo and HRS 812 as compared with the other three rootstocks (Table 2). Higher N were found in Nova mandarin grafted to HRS 812 and HRS 896 (~2.6%) versus the other rootstocks (~2.3%). A lower concentration of P was observed for Rough lemon (0.105 mg $kg^{-1}$) versus the other four rootstocks (~0.125 mg $kg^{-1}$). However, trees grafted to Rough lemon had higher concentrations of Mg and Mn than the other rootstocks (Table 2). No statistical differences were found for the other elements in Nova mandarin grafted to the five different rootstocks (Table 2).

Even though no statistical differences were observed for Al, Bo, Cu, Fe, and Zn, all of these elements were measurable in samples collected from both experimental orchards. In both orchards Al, Bo, Cu, Fe, and Zn elements ranged between 77–102, 47–68, 5.5–10, 72–143, and 25–34 mg $kg^{-1}$, respectively (data not shown).

### *3.3. Tree Response*

Rootstock type also appeared to affect tree height, tree diameter, TCV (Table 3), and tree efficiency in Tahiti lime (Figure 1A). When grafted to Carrizo, Cleopatra, or Rough lemon rootstocks, increases in tree height, tree diameter, and TCV were observed when compared to Swingle and HRS 812 rootstocks. In addition, almost twice the tree efficiency was found with Tahiti lime grafted to Swingle (35.6 fruits $m^{-3}$) versus Carrizo (18.7 fruits $m^{-3}$) rootstock. No statistical differences were found for total fruit number or for fruit weight for Tahiti lime scions grafted to all rootstocks, and none were found for any of the Nova mandarin tree variables and fruit production (Table 3, Figure 1B).

**Table 1.** Soil nutrients in two citrus orchards grown in Coto series grafted in five different rootstocks in the Agricultural Experiment Substation of Isabela, Puerto Rico.

| Scion | Rootstocks | OM $^{z}$ | pH | Ca | K | Mg | Na | P | S | $NO_3$-N |
|---|---|---|---|---|---|---|---|---|---|---|
| | | -%- | -1:1- | ------------------------- mg kg$^{-1}$ --------------------- | | | | | | -ppm- |
| Tahiti lime | Carrizo | 5.93 | 5.18 | 485 | 184 | 90.8 $^{ay}$ | 16.8 $^{a}$ | 15.6 | 80.3 $^{c}$ | 11.0 $^{b}$ |
| | Cleopatra | 5.40 | 5.00 | 521 | 258 | 74.0 $^{b}$ | 13.8 $^{ab}$ | 14.0 | 139 $^{a}$ | 28.5 $^{a}$ |
| - | HRS 812 | 5.58 | 4.85 | 360 | 230 | 54.3 $^{c}$ | 11.3 $^{b}$ | 21.0 | 146 $^{a}$ | 20.0 $^{a}$ |
| - | Rough lemon | 5.40 | 5.18 | 534 | 189 | 69.5 $^{b}$ | 11.5 $^{b}$ | 15.0 | 105 $^{b}$ | 10.5 $^{b}$ |
| - | Swingle | 5.53 | 5.30 | 485 | 218 | 76.0 $^{b}$ | 12.3 $^{ab}$ | 15.5 | 82.3 $^{c}$ | 9.0 $^{b}$ |
| - | Pr > F | 0.60 | 0.76 | 0.65 | 0.50 | 0.09 | 0.04 | 0.39 | 0.08 | 0.03 |
| Nova mandarin | Carrizo | 5.05 $^{a}$ | 4.70 | 486 | 272 | 77.8 | 10.0 $^{b}$ | 22.0 | 86.0 | 14.5 |
| | HRS 812 | 4.65 $^{b}$ | 4.95 | 501 | 206 | 81.0 | 10.5 $^{ab}$ | 13.5 | 83.5 | 16.5 |
| - | HRS 896 | 4.55 $^{b}$ | 4.83 | 506 | 240 | 84.5 | 14.8 $^{a}$ | 19.3 | 62.8 | 15.3 |
| - | Rough lemon | 4.53 $^{b}$ | 4.80 | 432 | 289 | 81.8 | 10.5 $^{ab}$ | 12.3 | 104 | 20.5 |
| - | Swingle | 4.58 $^{b}$ | 4.93 | 502 | 232 | 81.5 | 11.3 $^{ab}$ | 13.8 | 69.8 | 14.5 |
| | Pr > F | 0.09 | 0.85 | 0.90 | 0.13 | 0.99 | 0.09 | 0.57 | 0.66 | 0.64 |

$^{y}$ Means followed by the same letter in a column for each soil are not significantly different by Tukey's test at $p < 0.10$. $^{z}$ OM = organic matter, Ca = exchangeable calcium, K = exchangeable potassium, Mg = exchangeable magnesium, Na = exchangeable sodium, P = available phosphorous, S = total sulphur, and $NO_3$-N = nitrate.

**Table 2.** Mean concentrations of plant nutrients in two citrus orchards grown in Coto series grafted to five different rootstocks planted at the Agricultural Experiment Substation of Isabela, Puerto Rico.

| Scion | Rootstocks | N $^{z}$ | Ca | K | Mg | Na | P | S | Mn |
|---|---|---|---|---|---|---|---|---|---|
| - | - | ---%--- | ----------------------------------------- mg kg$^{-1}$ -------------------------------------- | | | | | | |
| **Tahiti** | Carrizo | 2.65 $^{aby}$ | 3.16 | 1.56 $^{b}$ | 0.360 $^{ab}$ | 0.030 | 0.158 | 0.255 | 48.3 $^{b}$ |
| **lime** | Cleopatra | 2.48 $^{b}$ | 3.04 | 1.59 $^{b}$ | 0.383 $^{a}$ | 0.030 | 0.138 | 0.223 | 101 $^{a}$ |
| - | HRS 812 | 2.67 $^{ab}$ | 3.07 | 1.60 $^{b}$ | 0.325 $^{abc}$ | 0.038 | 0.155 | 0.268 | 66.0 $^{ab}$ |
| - | Rough lemon | 2.49 $^{b}$ | 3.06 | 1.83 $^{a}$ | 0.303 $^{bc}$ | 0.028 | 0.150 | 0.213 | 74.8 $^{ab}$ |
| - | Swingle | 2.88 $^{a}$ | 2.74 | 1.76 $^{a}$ | 0.275 $^{c}$ | 0.033 | 0.160 | 0.245 | 71.8 $^{ab}$ |
| - | Pr > F | **0.05** | 0.64 | **0.09** | **0.003** | 0.29 | 0.50 | 0.51 | **0.03** |
| **Nova** | Carrizo | 2.29 $^{b}$ | 2.46 $^{a}$ | 1.20 | 0.282 $^{ab}$ | 0.145 | 0.130 $^{a}$ | 0.213 | 69.8 $^{b}$ |
| **mandarin** | HRS 812 | 2.60 $^{a}$ | 2.74 $^{a}$ | 1.13 | 0.295 $^{ab}$ | 0.135 | 0.130 $^{a}$ | 0.245 | 113 $^{ab}$ |
| - | HRS 896 | 2.62 $^{a}$ | 2.15 b | 1.27 | 0.233 $^{c}$ | 0.123 | 0.128 $^{a}$ | 0.208 | 93.5 $^{b}$ |
| - | Rough lemon | 2.33 $^{b}$ | 2.29 b | 1.06 | 0.315 $^{a}$ | 0.160 | 0.105 $^{b}$ | 0.215 | 165 $^{a}$ |
| - | Swingle | 2.31 $^{b}$ | 2.22 b | 1.24 | 0.243 $^{bc}$ | 0.123 | 0.123 $^{a}$ | 0.230 | 121 $^{ab}$ |
| - | Pr > F | **0.05** | **0.03** | 0.77 | **0.007** | 0.22 | **0.08** | 0.32 | **0.007** |

$^{z}$ N = nitrogen, Ca = calcium, K = potassium, Mg = magnesium, Na = sodium, P = phosphorous, S = sulfur, and Mn = manganese. $^{y}$ Means followed by the same letter in a column for each soil are not significantly different by Tukey's test at $p < 0.10$.

**Table 3.** Tree variables and fruit production of two citrus varieties grafted to five different rootstocks planted at the Agricultural Experiment Substation of Isabela, Puerto Rico from April 2015–May 2016.

| Scion | Rootstocks | Total Fruit Number | Total Fruit Weight | Tree Height | Tree Diameter | Tree Canopy Volume |
|---|---|---|---|---|---|---|
| | | **-# tree-** | **---kg---** | **--m--** | **--m$^2$--** | **--m$^3$--** |
| **Tahiti lime** | Carrizo | 684 | 19.3 | 3.6 $^{ay}$ | 21.2 $^a$ | 41.8 $^a$ |
| | Cleopatra | 1032 | 18.6 | 3.7 $^a$ | 22.4 $^a$ | 44.6 $^a$ |
| - | HRS 812 | 532 | 6.12 | 2.7 $^c$ | 14.8 $^c$ | 23.0 $^c$ |
| - | Rough lemon | 797 | 20.4 | 3.5 $^a$ | 19.1 $^b$ | 37.0 $^b$ |
| - | Swingle | 930 | 8.07 | 3.0 $^b$ | 16.1 $^c$ | 26.1 $^c$ |
| - | Pr > F | 0.330 | 0.230 | **0.016** | **0.039** | **0.015** |
| **Nova mandarin** | Carrizo | 13.0 | 1.51 | 2.22 | 4.06 | 4.76 |
| | HRS 812 | 11.5 | 1.24 | 2.42 | 4.27 | 5.59 |
| - | HRS 896 | 9.63 | 1.46 | 2.38 | 4.97 | 6.42 |
| - | Rough lemon | 12.0 | 1.56 | 2.13 | 4.53 | 5.48 |
| - | Swingle | 14.5 | 1.79 | 2.39 | 4.57 | 5.75 |
| - | Pr > F | 0.964 | 0.970 | 0.17 | 0.56 | 0.388 |

[y] Means followed by the same letter in a column for each variable are not significantly different by Tukey's test at $p < 0.10$.

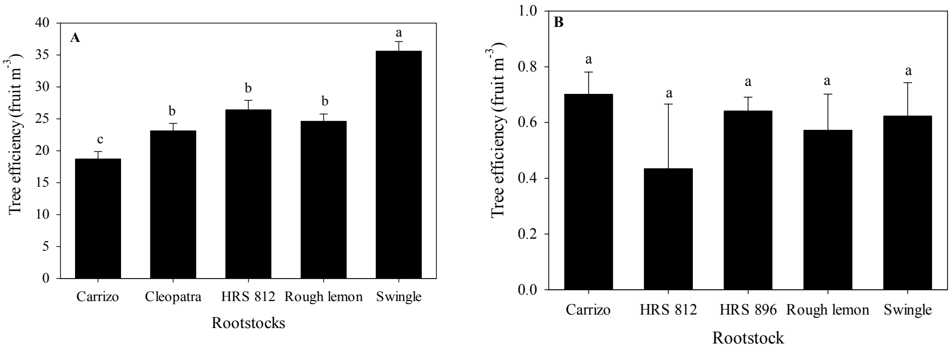

**Figure 1.** Tree efficiency (fruits $m^{-3}$) of (**A**) Tahiti lime and (**B**) Nova mandarin grafted to five different rootstocks planted at the Agricultural Experiment Substation of Isabela, Puerto Rico from April 2015–May 2016. Vertical lines represent the standard errors and lower case letters are different if there were statistical differences among the rootstocks by Tukey's test ($p < 0.10$) for each citrus species.

## 4. Discussion

The ideal soil pH for citrus trees ranges from slightly acidic soil (6) to alkaline (8). Soil acidity (pH less than 6), as found in Coto soils in Isabela, is well known as a major factor resulting in low crop yields due to Al and Mn toxicity, low concentrations of Ca and Mg, and decreasing availability of other nutrients like P [30]. Junior et al. [30] emphasized most of the response of citrus to liming agents was due to a high demand for Ca, used to regulate many processes related to both growth and responses to environmental stresses [31]. Also, liming with dolomitic limestone can satisfy the Mg demand.

Based on our results (Table 2 and data not shown (i.e., for Al, Bo, Cu, Fe, and Zn elements)), all the nutrients and elements were below the adequate ranges established by Mills and Jones [32] for lime. Although, for Nova mandarin, lower N (i.e., expected—3.0–3.5% versus the present study at 2.3–2.6%) and P (i.e., expected—0.15–0.25 versus this study <0.15) values observed were suggested by Mills and Jones [32] for mandarins.

When compared with previous results reported by Román-Pérez et al. [22] for the same orchards, the Tahiti lime trees yielded twice the number of fruit (797 v. 424), while mean fruit weight was less than half what they observed (20.4 g v. 42.7 g). For Nova mandarin orchard, quite low fruit production (~11 fruit/tree) was obtained due to HLB, most of them dried with thick skin. It is well established that HLB affects fruit production, resulting in smaller, evergreen, and dried fruit with an undesirable shape [33]. HLB can also cause severe tree deterioration until it dies [34]. Tree mortality by April 2016 was 13% for Tahiti lime grafted to HRS 812, Carrizo, and Rough lemon rootstocks, and 25% for Nova mandarin tree on Rough lemon and Carrizo rootstocks. Unfortunately, final *CLas* titers were not determined, as the values could have been a very useful parameter for further explaining rootstock performance.

Although the HLB caused some Tahiti lime tree mortality, Tahiti lime grafted to HRS 812 exhibited greater tree growth (height and diameter), developed greater canopy volume, and had higher tree efficiency as compared with trees on Cleopatra. However, studies driven by Piña et al. [35] found the opposite response of Tahiti lime growing in Fluventic Haplustolls soils in Venezuela and grafted to 11 different rootstocks. Also, even though our study had 13% tree mortality for Tahiti lime trees after 2013, compared with data collected in 2012–2013 by Román-Pérez et al. [33], there was a 3-fold increase in tree efficiency of Tahiti limes grafted to Carrizo, Cleopatra, HRS 812, and Rough lemon rootstocks in our study. In both studies, similar tree efficiency was found for limes on Swingle rootstock (extasciitilde 16.9 fruits $m^{-3}$). Meanwhile, in the Nova mandarin orchard, tree efficiency was less than 1.

## 5. Conclusions

Even though common management strategies recommended to reduce the effect of HLB are used, we have observed different tree responses based on different scion-rootstock combinations. The Tahiti lime growing in Coto soils in the northwest of Puerto Rico had benefits for tree growth parameters when grafted on Carrizo and Cleopatra rootstocks. However, tree efficiency for Tahiti lime grafted to Swingle was superior to that of other rootstocks. Based on our Tahiti lime tissue results, our fertilization program covered the orchard needs and supplied most of the nutrient needs for the Nova mandarin orchard. Given the prevalence of HLB at Isabela, and our observations of higher mortality, poor development, and low yield, we do not recommend future use of Nova mandarin there.

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