

Performance of HLB-affected Trees to Soil Macro- and Micronutrient Applications

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The huanglongbing (HLB) causative agent, Candidatus Liberibacter asiaticus, lowers tree performance by reducing water and nutrient uptake as a result of root loss. HLB-affected trees have a fibrous root loss of about 30 to 80%, which increases as HLB symptoms develop in the canopy. Investigating optimal nutrient concentrations in citrus roots thus improves our understanding of HLB dynamics concerning root nutrition and fertilizer application methods. This study sought to evaluate nutrient uptake of HLB-affected orange trees via soil fertilizer applications for 5- to 6-year-old Citrus sinensis 'Valencia' orange trees on Swingle rootstock at Ridge and Flatwoods sites. Macronutrients and micronutrients were applied at varying fertilization rates of standard fertilization via fertigation according to the University of Florida Institute of Food and Agricultural Science guidelines. For macronutrients, the rates were: a) standard fertilization + 40 lb/acre Ca + 40 lb/acre Mg + 220 lb/acre K and b) standard fertilization + 90 lb/acre Ca + 90 lb/acre Mg + 440 lb/ acre K. For micronutrients, the rates were: a) standard fertilization + 5 lb/acre Fe, 5 lb/acre Mn, 5 lb/acre Zn+1 lb/acre B; b) standard fertilization + 10 lb/acre Fe, 10 lb/acre Mn, 10 lb/acre Zn + 2 lb/acre B; and c) standard fertilization + 20 lb/acre Fe, 20 lb/acre Mn + 20 lb/acre Zn + 4 lb/acre B. Soil and leaf samples were collected for nutrient concentration analysis in Spring and Fall 2019 and Summer 2020. No significant differences among treatments (P < 0.05) were observed for tissue and soil nutrient concentrations due to nutrient interactions. Fruit yield between the 2019 and 2020 harvest seasons increased with increased nutrient availability. Therefore, at higher fertilization rates of (standard fertilization + 40 lb/acre Ca + 40 lb/acre Mg + 220 lb/acre K + 20 lb/acre Fe, 20 lb/acre Mn + 20 lb/acre Zn + 4 lb/acre B), HLB-affected trees showed increased nutrient uptake, improving overall tree performance.

Florida citrus production has been on the decline for the past two decades, with orange production declining by 72%, from about 8 to 2 billion tons from 2007–2008 to 2017–2018 (National Agricultural Statistics Service, 2020). Citrus production area in Florida has also declined from over 750,000 acres in 2000 to approximately 392,515 acres in 2019 (National Agricultural Statistics Service, 2020). The decline in citrus production is mainly due to citrus greening and damages from the Hurricane Irma in 2017 (Dala-Paula et al., 2019).

Citrus greening (also known as huanglongbing or HLB), is a disease caused by a bacterium *Candidatus* Liberibacter asiaticus (*CLas*), which lowers tree performance due to reduced uptake and accumulation of water and nutrients (Kadyampakeni et al., 2014). The decline in uptake and accumulation of water and nutrients is due to an alteration of the plant's photosynthesis mechanism, root length density and the vascular system (Graham et al., 2013). Symptoms of HLB can be detected on several parts of the plant, from roots to leaves as well as increased acidity and bitterness of the fruit, thus changing the chemical and sensory characteristics of the fruit (Bassanezi et al., 2009; Bové, 2006). HLB-affected trees have a reduced canopy, leaves show chlorotic patterns and fruit size is reduced. The fruit contains small, brownish, aborted

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seeds that can be seen when the orange fruit is sectioned perpendicularly to the fruit axis. The presence of *CLas* pathogen in a plant causes the fruits to drop prematurely causing a 30 to 100% yield reduction, resulting in fruit losses of approximately \$150 million annually (Gottwald et al., 2007).

Management strategies for HLB-affected trees include preventing the spread of infection by vector control and eliminating affected trees, while management of affected trees include pH regulation and foliar spray of readily absorbable nutrients and phytohormones to improve nutrient uptake (Dala-Paula et al., 2019). HLB-affected trees have a poorly developed and damaged root system due to fibrous root density loss of about 30 to 50%, which increases as HLB symptoms develop in the canopy. Thus, there is reduced nutrient uptake by the plants (Johnson and Graham, 2015).

The development of a proper nutrition program for citrus trees is important as it provides the essential elements required by the trees for maintenance, improved yield, and fruit quality (Aular et al., 2017). For green plants to function and grow well, 17 elements are essential, and among these, oxygen (O), carbon (C), and hydrogen (H) are freely abundant in nature (Havlin et al., 2014). The 14 other mineral elements are divided into macronutrients and micronutrients. Macronutrients are elements that a plant requires in large quantities while micronutrients are required in small quantities. Macronutrients include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and micronutrients include iron (Fe), zinc (Zn),

manganese (Mn), boron (B), copper (Cu), molybdenum (Mo), nickel (Ni), and chlorine (Cl) (Havlin et al., 2014; Timilsena et al., 2014; Barker and Pilbeam, 2015; Zekri and Obreza, 2016). When an essential element is deficient, tree performance declines. Nutrient deficiencies result in distinct symptoms that can be exhibited in the leaves, twigs, and fruits while an excessive amount of an essential element can lead to toxicity, which hinders tree performance (Obreza and Morgan, 2008).

Potassium is an important macronutrient that helps in sugar and starch metabolism and protein synthesis (Abbas and Fares, 2009). Potassium is essential in citrus production because it helps improve fruit size, fruit number and fruit quality (Aly et al., 2015). K deficiency in citrus causes slow vegetative growth, which leads to reduced fruit number, thinning of foliage, fruit drop, and fruit splitting (Ritenour et al., 2003; Obreza and Morgan, 2008; Zekri and Obreza, 2016). Excess K content causes a decrease in juice quality because of a decrease in fruit total soluble solids, juice content, juice color, and sugar to acid ratio of the citrus (Ritenour et al., 2003). Sources of K in intensive citrus production systems in Florida are potassium chloride (KCl), potassium sulfate and potassium nitrate, with KCl having the greatest consumption in the US (Havlin et al., 2014).

Calcium is an essential nutrient in citrus production that helps to strengthen the cell wall structure and aids in root development and functioning (Crowley, 2012; Havlin et al, 2014). An adequate supply of Ca is essential in promoting tree growth and fruit development (Zekri and Obreza, 2016). An insufficient supply of Ca causes poor nutrient and water uptake due to low carbohydrate content in the roots, resulting in reduced plant growth and fruit yield (Havlin, et al, 2014; Zekri and Obreza, 2016).

Iron is an essential element in plant nutrition because of its vital role in nitrogen fixation (Srivastava, 2013). Iron availability is affected by phosphorus heavy metals. High P content and accumulation of heavy metals such as Cu cause Fe deficiency in the soil. Fe deficiency can also be induced by Zn and Mn deficiencies (Zekri and Obreza, 2016).

Zinc is important for the formation of auxins that promote growth in plants, the formation of chlorophyll, plant carbon metabolism and improving water uptake by plants (Hansch and Mendel 2009). Thus, an inadequate supply of Zn results in decreased plant growth, stress tolerance and chlorophyll synthesis (Kawachi et al. 2009). Thus, Zn fertilization is vital because it increases the availability of the nutrient in the soil and the content in the orange trees (Hippler, 2015).

Currently, there are no clear guidelines for determining optimal nutrient concentrations in citrus roots to understand the relationship between root nutrition and HLB-affected trees for improved management strategies (Morgan et al., 2006; Eissenstat, 1991; Castle and Krezdorn, 1975). Therefore, investigating optimal nutrient concentrations in citrus roots would help to improve our understanding of the dynamics of HLB concerning root nutrition and fertilizer application methods, most importantly fertigation and soil application. This study was conducted to evaluate nutrient uptake of HLB-affected orange trees because of differential macro- and micronutrient fertilization. The specific objectives were: 1) to determine optimal nutrient concentrations in the soil and leaves for orange trees and 2) compare soil fertilization rates to identify optimal fertilizer rate for nutrient uptake into both underground and above ground components. The study hypothesized that higher soil nutrient, and fertilization rates would result in optimal nutrient concentrations for improved tree health in HLB-affected orange trees and higher rates of micronutrient

fertilization guidelines would improve root health of HLB affected orange trees, thereby improving the overall performance of the orange trees.

Materials and Methods

SITE DESCRIPTION. The study was conducted at the Citrus Research and Education Center (CREC), Lake Alfred, FL. (28°06'28.6"N; 81°41'07.8"W) and on a Flatwoods site near Clewiston, FL. (N 26°44'20.851"; W-81°4'54.568") to determine optimal fertigation and fertilization schemes. The two sites have 5- to 6-year-old Citrus sinensis 'Valencia' orange trees on Swingle rootstock. The Ridge soils at CREC site are Entisols classified as hyperthermic, uncoated lamellic quartzipsamments family (United States Department of Agriculture: Natural Resources Conservation Service, 2013). These soils are excessively drained and formed from eolian deposits and sandy marine deposits. The slope of the Ridge soils is 0 to 5% (United States Department of Agriculture: Natural Resources Conservation Service, 2019). Soils at the southwest Flatwoods site are Entisols of the siliceous, hyperthermic family of Mollic Psammaquents. The soils are poorly drained, rapidly permeable soils that are formed in sandy marine sediment underlain by limestone. The slope of these soils is 0 to 2% (United States Department of Agriculture: Soil Conservation Service, 1990). The Ridge soils have a high density of trees of about 1359 trees/ha while the Flatwoods soils of southwest Florida have a lower tree density of about 716 trees/ha.

EXPERIMENTAL DESIGN. The experimental design was a randomized complete block factorial design with an evaluation of macronutrients K and Ca at: a) 220 lb/acre K and 40 lb/acre Ca (1× macronutrients) and b) 440 lb/acre K and 90 lb/acre Ca (2× macronutrients); and micronutrients (Zn and Fe) at: a) 5 lb/acre (1× micronutrients), b)10 lb/acre (2× micronutrients), and c) 20 lb/acre (4× of micronutrients) of the current University of Florida Institute of Food and Agricultural Science (UF IFAS) fertilization guidelines (Obreza and Morgan, 2008; Morgan and Kadyampakeni, 2020). Macronutrients and micronutrients were applied three times per year on the soil. Each plot had 10 trees where the middle 8 trees were the experimental unit. All treatments were replicated 6 times.

The treatments were as follows:

- Control with standard fertilization via fertigation of N, P, S, Mo, and Cu fertilization according to UF IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.
- Standard fertilization + 1X macronutrient (MA) + 1× micronutrient (MI) (soil applied).
- Standard fertilization + $1 \times MA + 2 \times MI$ (soil applied).
- Standard fertilization + $1 \times MA + 4 \times MI$ (soil applied).
- Standard fertilization + $2 \times MA + 1 \times MI$ (soil applied).
- Standard fertilization + $2 \times MA + 2 \times MI$ (soil applied).
- Standard fertilization $+ 2 \times MA + 4 \times MI$ (soil applied).

SAMPLING METHODS. To determine optimal nutrient concentrations in the soil and leaves for orange trees, leaf and soil nutrient concentrations were evaluated in May and Nov. 2019 and July 2020. About 20 mature leaves were collected at each time point in the northwest, southwest, northeast, and southeast directions with reference to the sampled orange tree. Soil samples were collected at a depth of 0–15 cm. Soil and leaf samples were processed for nutrient content analysis.

DATA ANALYSIS. Data analyses to evaluate if there are treatment, synergistic or interaction effects among macro- and micronutrient application rates and relationships between fertilizer application rates and nutrient content were done using R version 4.0.2 (R Core Team, 2013). The variates evaluated were plant tissue and soil concentrations of K, Ca, Fe, and Zn. Analysis of variance (ANOVA) was done to compare treatments once the ANOVA assumptions such as normality, homogeneity of variance and uniform distribution of the data, were met. Tukey's Honestly Significant Difference (HSD) test was conducted to evaluate the means that were significantly different from the others based on the variates under evaluation.

Results

PLANT TISSUE NUTRIENT CONCENTRATIONS. There was a gradual increase in K concentrations from May 2019 to July 2020 (Table 1). No significant differences were obtained in K concentrations of the leaf tissues among treatments (ANOVA, P = 0.05). Trees that received Treatment 5 showed the highest K concentration in July 2020 and Treatment 6 had the lowest leaf K concentration (Table 1). Leaf Ca concentrations decreased from May 2019 to July 2020. There were no significant differences in Ca concentrations among treatments throughout the period of study. Treatment 7 had the greatest leaf Ca concentrations, followed by Treatments 1, 4, and 6, while Treatment 5 had the least leaf Ca concentration. The results of the study showed that for all treatments, there was an increase in Zn concentrations at Flatwoods site in July 2020 as compared to May 2019 when the project had just started (Table 2). However, the results from these trials show no significant differences in Zn plant tissue concentrations among all the treatments (P > 0.05). Zinc concentrations in plant tissue ranged between 40.17 ± 8.42 ppm and 48.67 ± 4.80 ppm. Iron exhibited a different trend in its concentrations throughout the

period of study, which show a reduction in Fe concentrations in Nov. 2019 as compared to May 2019. Nevertheless, there was an increase in the Fe concentrations in plant tissues in July 2020. The increase in Fe concentrations in plant tissues did not differ significantly among the treatments (P > 0.05). Treatment 4 had the highest Fe concentration while Treatment 2 had the least Fe concentration.

Potassium concentrations decreased sharply in Nov. 2019 at the Ridge site, and gradually increased in July 2020 for all treatments (Table 3). No significant differences among treatments (P > 0.05) were observed. There was an increase in Ca concentrations at Ridge site in Nov. 2019 as compared to May 2019. However, leaf Ca concentrations decreased in July 2020. There were no significant differences in leaf Ca concentrations among treatments. At the end of the trial, Treatment 7 had the greatest leaf Ca concentration while Treatment 2 had the lowest Ca concentration. Results further showed that for all treatments. there was an increase in Zn concentrations at Ridge site in July 2020 in comparison to May 2019 (Table 4). However, the results obtained did not have significant differences among all the treatments (P > 0.05). Concentrations of Zn ranged between 28.67 \pm 4.23 ppm and 33.67 \pm 4.72 ppm. Leaf tissue concentrations of Fe decreased greatly in July 2020 as compared to May 2019. In July 2020, the control (Treatment 1) had the highest Fe concentration of 45.50 ± 2.51 ppm while Treatment 6 had the lowest Fe concentration of 38.00 ± 4.82 ppm.

SOIL NUTRIENT CONCENTRATIONS. A trend similar to that of leaf tissue nutrient concentrations was observed for nutrient concentrations in the soil. Soil K concentrations at the Flatwoods site increased from May 2019 to Nov. 2019 (Table 5). There were no significant differences among treatments for soil K concentrations. The concentration of K in the soil ranged between 54 ± 21 mg kg⁻¹and 83 ± 36 mg kg⁻¹. Soil Ca concentrations decreased between May 2019 and Nov. 2019. The concentration of Ca

Table 1. Potassium (K) and calcium (Ca) uptake in the leaves of *Citrus sinensis* 'Valencia' orange trees as a function of differential fertilizer application rates at a Flatwoods site in central Florida.

		K			Ca	
Treatment	May 2019	Nov. 2019	July 2020	May 2019	Nov. 2019	July 2020
1z	1.36 ± 0.36^{y}	1.61 ± 0.10	1.76 ± 0.10	4.29 ± 0.50	4.04 ± 0.42	3.97 ± 0.59
2 ^x	1.38 ± 0.27	1.63 ± 0.18	1.78 ± 0.11	4.07 ± 0.38	3.94 ± 0.35	3.87 ± 0.26
3^{w}	1.49 ± 0.11	1.46 ± 0.10	1.76 ± 0.09	4.11 ± 0.32	4.14 ± 0.46	3.96 ± 0.49
4v	1.44 ± 0.33	1.63 ± 0.12	1.82 ± 0.09	4.08 ± 0.40	3.81 ± 0.19	3.94 ± 0.30
5 ^u	1.66 ± 0.09	1.49 ± 0.12	1.85 ± 0.11	3.84 ± 0.22	4.09 ± 0.55	3.72 ± 0.22
6^{t}	1.51 ± 0.17	1.54 ± 0.19	1.75 ± 0.15	4.19 ± 0.41	3.88 ± 0.47	3.72 ± 0.26
7s	1.55 ± 0.14	1.62 ± 0.15	1.77 ± 0.08	3.97 ± 0.34	3.98 ± 0.44	3.98 ± 0.13
P-value	0.34 ns	0.22 ns	0.58 ns	0.51 ns	0.82 ns	0.71 ns

²Control with standard fertilization via fertigation of N, P, S, Mo, Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

^y Means \pm SD followed by different lowercase letters which are significantly different at P < 0.05 by Tukey's HSD test.

^{*}Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

 $^{^{}w}$ Standard fertilization + 1× MA + 2× MI (soil applied).

 $^{^{}v}$ Standard fertilization + 1× MA + 4× MI (soil applied).

^uStandard fertilization + $2 \times MA + 1 \times MI$ (soil applied).

Standard fertilization $+2 \times MA + 1 \times MI$ (soil applied).

^sStandard fertilization + 2× MA + 4× MI (soil applied).

ns = nonsignificant at P < 0.05 by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2× and 4× MI refer to 5.6, 11.2 and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn and Zn, and 1-, 2-, and 4-lb/acre of B per year.

ranged between 793 \pm 224 mg $kg^{\text{-}1}$ and 1335 \pm 832 mg $kg^{\text{-}1}$. No significant differences were observed among treatments for Ca concentrations in the soil. Soil Zn concentration increased between May 2019 and Nov. 2019 (Table 6). No significant differences among treatments were observed for Zn concentrations. Treatment 7 had the highest Zn concentration of 38 \pm 23 mg $kg^{\text{-}1}$ while Treatment 2 had the least Zn concentration of 19 ± 7 mg $kg^{\text{-}1}$. Generally, there was a decrease in Fe concentration from

May 2019 to Nov. 2019. Soil Fe concentrations ranged between 126 ± 24 mg kg⁻¹ and 147 ± 26 mg kg⁻¹ (Table 6).

Similarly, soil K concentrations at Ridge site decreased from May 2019 to July 2020 (Table 7). The difference in K concentrations among treatments was not significant in July 2020. Treatment 2 had the highest K concentration of 32 ± 11 mg kg⁻¹ while Treatment 1

Table 2. Zinc (Zn) and iron (Fe) uptake in the leaves of *Citrus sinensis* 'Valencia' orange trees as a function of differential fertilizer application rates at a Flatwoods site in central Florida.

		Zn			Fe	
Treatment	May 2019	Nov. 2019	July 2020	m May 2019	Nov. 2019	July 2020
1z	$22.54 \pm 3.68^{\text{y}}$	29.00 ± 4.47	44.50 ± 9.85	61.77 ± 6.26	58.67 ± 6.86	66.30 ± 14.10
2x	22.47 ± 3.11	28.67 ± 2.58	40.17 ± 8.42	60.11 ± 7.26	54.33 ± 2.73	64.67 ± 13.29
3^{w}	26.06 ± 3.43	28.00 ± 2.90	46.33 ± 9.29	65.06 ± 11.38	57.50 ± 6.22	71.50 ± 9.40
4v	23.73 ± 2.02	28.83 ± 4.92	48.67 ± 4.80	66.25 ± 4.26	63.50 ± 17.21	72.50 ± 9.61
5 ^u	26.48 ± 2.79	27.00 ± 2.00	44.50 ± 4.89	63.11 ± 10.48	51.17 ± 4.49	63.67 ± 6.59
6t	25.06 ± 4.95	28.33 ± 5.16	42.50 ± 6.09	61.64 ± 3.83	58.33 ± 10.56	70.17 ± 12.37
7s	23.51 ± 3.36	27.83 ± 2.48	42.83 ± 9.87	63.44 ± 4.46	57.50 ± 3.02	71.33 ± 14.22
P-value	0.27 ns	0.97 ns	0.63 ns	0.81 ns	0.35 ns	0.75 ns

²Control with standard fertilization via fertigation of N, P, S, Mo, and Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

ns = nonsignificant at P < 0.05 by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2v, and 4v MI refer to 5.6, 11.2, and 22.4 kg/ha (or 5, 10. and 20 lb/acre) of micronutrients Fe, Mn, and Zn, and 1-, 2- and 4-lb/acre of B per year.

Table 3. Potassium (K) and calcium (Ca) uptake in the leaves of *Citrus sinensis* 'Valencia' orange trees as a function of differential fertilizer application rates at a Ridge site in central Florida.

		K			CA		
			[pm			
Treatment	May 2019	Nov. 2019	July 2020	May 2019	Nov. 2019	July 2020	
1 z	1.71 ± 0.12^{y}	1.30 ± 0.11	1.31 ± 0.1	3.56 ± 0.10	3.85 ± 0.27	3.16 ± 0.12	
2×	1.67 ± 0.10	1.21 ± 0.13	1.50 ± 0.07	3.41 ± 0.22	4.26 ± 0.40	3.08 ± 0.20	
3w	1.69 ± 0.11	1.24 ± 0.28	1.39 ± 0.19	3.28 ± 0.25	4.21 ± 0.33	3.23 ± 0.22	
4v	1.66 ± 0.11	1.30 ± 0.25	1.48 ± 0.16	3.30 ± 0.24	3.97 ± 0.33	3.09 ± 0.26	
5 ^u	1.69 ± 0.09	1.21 ± 0.09	1.39 ± 0.31	3.50 ± 0.24	4.14 ± 0.34	3.16 ± 0.1	
6^{t}	1.72 ± 0.07	1.26 ± 0.14	1.40 ± 0.23	3.25 ± 0.33	4.03 ± 0.25	3.24 ± 0.24	
7s	1.78 ± 0.14	1.20 ± 0.18	1.39 ± 0.24	3.25 ± 0.26	4.35 ± 0.30	3.27 ± 0.27	
P-value	0.62 ns	0.92 ns	0.72 ns	0.17 ns	0.13 ns	0.64 ns	

²Control with standard fertilization via fertigation of N, P, S, Mo, Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

 $^{^{}y}$ Means \pm SD followed by different lowercase letters which are significantly different at P < 0.05 by Tukey's HSD test.

^{*}Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

[&]quot;Standard fertilization + $1 \times MA + 2 \times MI$ (soil applied).

 $^{^{}v}$ Standard fertilization + 1× MA + 4× MI (soil applied).

 $^{{}^{\}text{u}}$ Standard fertilization + 2× MA + 1× MI (soil applied).

 $^{^{}t}$ Standard fertilization + 2× MA + 2× MI (soil applied).

^sStandard fertilization + 2× MA + 4× MI (soil applied).

 $^{^{}y}$ Means \pm SD followed by different lowercase letters which are significantly different at P < 0.05 by Tukey's HSD test

^{*}Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

 $^{^{\}text{w}}$ Standard fertilization + 1× MA + 2× MI (soil applied).

^vStandard fertilization + 1× MA + 4× MI (soil applied).

^uStandard fertilization + 2× MA + 1× MI (soil applied).

 $^{^{}t}$ Standard fertilization + 2× MA + 2× MI (soil applied).

^sStandard fertilization + 2× MA + 4× MI (soil applied).

ns = nonsignificant at P < 0.05 by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/per acre of K. 1×, 2× and 4× MI refer to 5.6, 11.2 and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn, and Zn, and 1-, 2-, and 4-lb/acre of B per year.

had the least K concentration of 29 ± 12 mg kg⁻¹. There was a reduction in Ca concentration from May 2019 to July 2020. Calcium concentration in the soil ranged between 603 ± 82 mg kg⁻¹ and 720 ± 6 mg kg⁻¹. Zinc concentration in the soil increased over time, from May 2019 to July 2020. No significant differences among treatments were observed for Zn concentrations; with Treatment 7 having the greatest Zn concentration of 60 ± 19 mg kg⁻¹ while Treatment 5 had the least K concentration of 43 ± 8

mg kg⁻¹ (Table 8). There was a reduction in Fe concentration in the soil from May 2019 to July 2020. Concentration of Fe ranged between 124 ± 20 mg kg⁻¹ and 144 ± 15 mg kg⁻¹.

FRUIT YIELD. There was an increase in fruit yield between 2019 and 2020 at the Ridge site ranging between 26.3 % and 55.8% (Table 9). Treatment 2 had the greatest yield in 2020 of 12.2 ± 1.5 t/ha while Treatment 5 had the least yield of 9.6 ± 2.9 t/ha. There was no comparison of yield data for Flatwoods

Table 4. Zinc (Zn) and Iron (Fe) uptake in the leaves of *Citrus sinensis* 'Valencia' orange trees as a function of differential fertilizer application rates at a Ridge site in central Florida.

		Zn		Fe		
Treatment	May 2019	Nov. 2019	July-2020	pm May 2019	Nov. 2019	July 2020
1 ^z	$19.41 \pm 4.26^{\text{y}}$	14.17 ± 2.56	30.67 ± 1.86	75.04 ± 6.75	59.67 ± 24.95	45.50 ± 2.51
2 ^x	21.22 ± 2.00	14.50 ± 1.76	28.83 ± 6.91	84.80 ± 6.22	45.33 ± 4.89	39.00 ± 2.28
3w	20.24 ± 2.56	14.33 ± 3.56	28.67 ± 4.23	72.51 ± 9.43	62.00 ± 18.22	41.00 ± 4.94
4v	19.64 ± 0.94	13.83 ± 1.83	33.67 ± 4.72	77.14 ± 7.99	45.17 ± 6.65	43.00 ± 4.52
5 ^u	20.37 ± 3.32	14.33 ± 1.03	29.50 ± 4.37	80.86 ± 10.99	48.17 ± 4.31	39.50 ± 6.53
6t	21.36 ± 2.17	15.17 ± 3.19	29.83 ± 4.88	79.67 ± 11.18	48.33 ± 6.35	38.00 ± 4.82
7s	20.80 ± 1.76	14.17 ± 1.83	28.83 ± 4.45	77.43 ± 12.80	57.33 ± 14.21	45.33 ± 7.55
P-value	0.82 ns	0.98 ns	0.54 ns	0.41 ns	0.16 ns	0.07 ns

²Control with standard fertilization via fertigation of N, P, S, Mo, Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

ns = nonsignificant at P < 0.05 by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2×, and 4× MI refer to 5.6, 11.2, and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn and Zn, and 1-, 2-, and 4-lb/per acre of B per year.

Table 5. Soil potassium (K) and calcium (Ca) concentrations as a function of differential fertilizer application rates at a Flatwoods site in central Florida.

	K		Ca		
т , ,	M 2010	mg/k	2	N. 2010	
Treatment	May 2019	Nov. 2019	May 2019	Nov. 2019	
1z	47.00 ± 11.82^{y}	59.13 ± 6.02	2334 ± 2024	1335 ± 832	
2×	49.67 ± 14.46	66.17 ± 39.14	2280 ± 3392	1309 ± 805	
3w	56.08 ± 27.92	65.25 ± 39.13	1147 ± 779	1215 ± 585	
4v	66.50 ± 26.59	58.13 ± 17.68	1505 ± 1231	793 ± 224	
5u	47.67 ± 24.93	67.96 ± 47.67	2131 ± 2067	1096 ± 565	
5 ^t	42.50 ± 13.77	54.08 ± 20.97	1104 ± 769	638 ± 336	
7s	43.75 ± 15.33	82.63 ± 35.71	1350 ± 949	837 ± 416	
P-value	0.43 ns	0.81 ns	0.79 ns	0.25 ns	

²Control with standard fertilization via fertigation of N, P, S, Mo, and Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

 $^{^{\}circ}$ Means \pm SD followed by different lowercase letters which are significantly different at P < 0.05 by Tukey's HSD test

^{*}Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

 $^{^{\}mathrm{w}}$ Standard fertilization + 1× MA + 2× MI (soil applied).

vStandard fertilization + $1 \times MA + 4 \times MI$ (soil applied).

^uStandard fertilization + 2× MA + 1× MI (soil applied)

 $^{^{}t}$ Standard fertilization + 2× MA + 2× MI (soil applied).

 $^{^{}s}$ Standard fertilization + 2× MA + 4× MI (soil applied).

 $^{^{}y}$ Means \pm SD followed by different lowercase letters which are significantly different at P < 0.05 by Tukey's HSD test.

^{*}Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

 $^{^{}W}$ Standard fertilization + 1× MA + 2× MI (soil applied).

 $^{^{}v}$ Standard fertilization + 1× MA + 4× MI (soil applied).

^uStandard fertilization + 2× MA + 1× MI (soil applied).

 $^{^{}t}$ Standard fertilization + 2× MA + 2× MI (soil applied).

 $^{^{\}rm s}$ Standard fertilization + 2× MA + 4× MI (soil applied).

ns = nonsignificant at P < 0.05 by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2×, and 4× MI refer to 5.6, 11.2 and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn and Zn, and 1-, 2- and 4-lb/acre of B per year.

site since no data collection for 2020 was done due to the COVID-19 lockdown.

Discussion

TISSUE NUTRIENT CONCENTRATIONS. The trend in calcium foliar concentrations can be due to interactions with K concentrations in the soil. Increased K concentrations in the soil cause a decrease in root uptake of Ca and Mg (Jakobsen, 1993). Thus, for the Flatwoods site, there was an increase in K concentrations

throughout the period of study, which had an antagonistic effect on Ca, resulting in reduced Ca concentrations. Similarly, for the Ridge site, Ca concentrations were relatively high, which resulted in reduced K concentrations in leaf tissues.

Calcium and Fe also have an antagonistic effect on Zn availability and translocation within the plant. At higher fertilization rates, there was an increase in Ca and Fe concentrations, which reduce the absorption of Zn by the roots and its translocation to the leaves (Prasad et al., 2016). Similar trends were also observed by Phuyal et. al (2020), whose study conducted on HLB-affected

Table 6. Soil zinc (Zn) and iron (Fe) concentrations as a function of differential fertilizer application rates at a Flatwoods site in central Florida.

	, , ,		11	
		Zn	I	Fe
		mg/k	кg	
Treatment	May 2019	Nov. 2019	May 2019	Nov. 2019
1 z	14.68 ± 7.92^{y}	32.75 ± 32.51	127.92 ± 41.03	138.92 ± 21.14
2 ^x	13.31 ± 8.39	19.21 ± 7.19	136.58 ± 54.45	125.50 ± 24.24
3 ^w	20.78 ± 16.09	22.40 ± 10.18	169.83 ± 25.86	157.54 ± 18.39
4v	17.32 ± 10.78	29.27 ± 13.68	126.17 ± 34.28	146.96 ± 25.95
5 ^u	20.71 ± 6.03	28.80 ± 7.03	151.33 ± 46.17	131.75 ± 12.99
6^{t}	11.26 ± 7.27	25.92 ± 7.03	150.17 ± 18.09	134.21 ± 36.82
7s	14.93 ± 7.69	37.45 ± 22.53	155.83 ± 50.31	132.29 ± 27.76
P-value	0.54 ns	0.58 ns	0.49 ns	0.36 ns

²Control with standard fertilization via fertigation of N, P, S, Mo, and Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

ns = nonsignificant at P < 0.05 by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2× and 4× MI refer to 5.6, 11.2, and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn and Zn, and 1-, 2-, and 4-lb/per acre of B per year.

Table 7. Soil potassium (K) and calcium (Ca) concentrations as a function of differential fertilizer application rates at a Ridge site in central Florida.

		K			Ca		
				mg/kg			
Treatment	May 2019	Nov. 2019	July 2020	May 2019	Nov. 2019	July 2020	
1z	73.08 ± 14.94 ^y	40.46 ± 6.57 b	24.75 ± 5.15	760.33 ± 102.29	596.92 ± 82.85	720.42 ± 96.12	
2×	62.25 ± 9.50	53.54 ± 18.00 ab	31.67 ± 10.87	786.50 ± 195.10	429.75 ± 141.64	616.17 ± 63.66	
3w	69.58 ± 10.22	$49.75 \pm 15.88 \text{ b}$	29.92 ± 9.47	811.83 ± 131.26	519.42 ± 109.06	811.08 ± 301.29	
4v	65.33 ± 13.63	61.04 ± 36.26 ab	29.67 ± 6.66	752.50 ± 263.53	452.50 ± 65.86	602.58 ± 82.36	
5 ^u	66.75 ± 15.01	51.88 ± 21.79 ab	30.42 ± 16.45	866.25 ± 97.13	486.63 ± 191.64	626.25 ± 98.58	
6t	68.25 ± 10.12	117.67 ± 74.72 a	28.67 ± 11.59	765.17 ± 156.75	527.25 ± 67.80	681.17 ± 150.07	
7s	71.92 ± 9.33	78.58 ± 40.49 ab	30.08 ± 6.51	880.75 ± 177.85	564.83 ± 87.21	711.33 ± 131.63	
P-value	0.74 ns	0.02*	0.94 ns	0.76 ns	0.17 ns	0.17 ns	

²Control with standard fertilization via fertigation of N, P, S, Mo, Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

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 $^{^{}y}$ Means \pm SD followed by different lowercase letters which are significantly different at P < 0.05 by Tukey's HSD test.

^{*}Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

wStandard fertilization + $1 \times MA + 2 \times MI$ (soil applied).

 $^{^{}v}$ Standard fertilization + 1× MA + 4× MI (soil applied).

[&]quot;Standard fertilization + $2 \times MA + 1 \times MI$ (soil applied).

 $^{^{}t}$ Standard fertilization + 2× MA + 2× MI (soil applied).

 $^{^{}s}$ Standard fertilization + 2× MA + 4× MI (soil applied).

 $^{^{}y}$ Means \pm SD followed by different lowercase letters which are significantly different at P < 0.05 by Tukey's HSD test.

^{*}Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

 $^{^{}W}$ Standard fertilization + 1× MA + 2× MI (soil applied).

^vStandard fertilization + 1× MA + 4× MI (soil applied).

^uStandard fertilization + 2× MA + 1× MI (soil applied).

 $^{^{}t}$ Standard fertilization + 2× MA + 2× MI (soil applied).

 $^{^{}s}$ Standard fertilization + 2× MA + 4× MI (soil applied).

ns, * = nonsignificant or significant at P < 0.05 by Tukey's HSD test, respectively. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2×, and 4× MI refer to 5.6, 11.2 and 22.4 kg/ha (or 5, 10, and 20 lb.acre) of micronutrients Fe, Mn and Zn, and 1-, 2-, and 4-lb/acre of B per year.

Table 8. Soil zinc (Zn) and iron (Fe) concentrations as a function of differential fertilizer application rates at a Ridge site in central Florida.

		Zn			Fe	_
			m	ng/kg		
Treatment	May 2019	Nov. 2019	July 2020	May 2019	Nov. 2019	July 2020
1z	$43.63 \pm 11.40^{\text{y}}$	40.45 ± 24.73	48.38 ± 7.73	141.42 ± 11.26	133.29 ± 10.61	129.75 ± 16.20
2×	42.43 ± 12.92	27.47 ± 7.81	45.63 ± 14.71	136.25 ± 11.04	136.00 ± 22.84	143.92 ± 14.84
3^{w}	45.52 ± 12.24	37.05 ± 14.94	55.60 ± 17.68	131.08 ± 15.18	132.46 ± 18.13	123.58 ± 19.99
4v	43.70 ± 16.04	59.74 ± 21.62	54.61 ± 10.26	133.67 ± 13.19	132.88 ± 9.51	137.50 ± 15.06
5 ^u	38.89 ± 7.83	28.69 ± 14.29	42.59 ± 7.46	131.25 ± 16.01	136.67 ± 28.33	142.25 ± 9.03
6t	45.15 ± 13.33	48.30 ± 15.48	55.23 ± 17.70	138.58 ± 11.51	132.75 ± 10.78	136.25 ± 15.90
7s	48.77 ± 12.80	52.18 ± 35.00	59.46 ± 18.91	136.42 ± 13.16	125.17 ± 14.69	128.00 ± 23.80
P-value	0.91 ns	0.09 ns	0.63 ns	0.80 ns	0.95 ns	0.92 ns

²Control with standard fertilization via fertigation of N, P, S, Mo, and Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

ns = nonsignificant at P < 0.05 by Tukey's HSD test. 1× and 2× MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. 1×, 2× and 4× MI refer to 5.6, 11.2, and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn and Zn, and 1-, 2- and 4-lb/acre of B per year.

Table 9. Fruit yield of *Citrus sinensis* 'Valencia' orange trees at Flatwoods and Ridge sites in central Florida as a function of differential fertilizer application rates (t/ha).

		Fruit yield (t	/ha)		
	Ridg	Ridge site			
Treatment	2019	2020	2019		
1 ^z	7.5 ± 3.0^{y}	11.5 ± 3.1	13.3 ± 1.5		
2x	8.0 ± 2.3	12.2 ± 1.5	13.8 ± 1.7		
3^{w}	7.8 ± 2.4	11.6 ± 3.4	13.5 ± 1.8		
4v	8.4 ± 3.5	13.0 ± 2.1	14.1 ± 2.7		
5 ^u	7.6 ± 2.0	9.6 ± 2.9	13.4 ± 2.7		
6^{t}	7.7 ± 2.6	12.0 ± 3.2	13.9 ± 3.3		
7s	8.3 ± 2.3	10.7 ± 3.3	13.0 ± 1.3		
P-value	0.995 ns	0.526 ns	0.984 ns		

²Control with standard fertilization via fertigation of N, P, S, Mo, Cu fertilization according to UF/IFAS guidelines. No extra K, Mg, Ca, Mn, Fe, B, and Zn.

^yMeans \pm SD followed by different lowercase letters are significantly different at P < 0.05 by Tukey's HSD test.

*Standard fertilization + $1 \times$ macronutrient (MA) + $1 \times$ micronutrient (MI) (soil applied).

ns = nonsignificant at P < 0.05 by Tukey's HSD test respectively. $1 \times$ and $2 \times$ MA refer to UF/IFAS recommendation of the 45 and 90 kg/ha (40 and 90 lb/acre) of macronutrients Ca and Mg and 220 and 440 lb/acre of K. $1 \times$, $2 \times$ and $4 \times$ MI refer to 5.6, 11.2, and 22.4 kg/ha (or 5, 10, and 20 lb/acre) of micronutrients Fe, Mn, and Zn, and 1 -, 2 - and 4 - lb/acre of B per year.

'Ray Ruby' grapefruit showed an increase in K and Zn due to interactions with other nutrients.

Soil nutrient concentrations. Nutrient interactions played a huge role in the availability of nutrients for orange tree uptake. An antagonistic effect between K and Ca was observed. An increase in K concentration in the soil resulted in a decrease in Ca at both the Flatwoods and Ridge sites. Calcium also competes with zinc for adsorption sites on soil particles and root particles. Thus, with increased Zn concentrations in the soil, there will be fewer adsorption sites for Ca resulting in reduced Ca concentrations. Additionally, interaction effects were observed between Zn and Fe. Zinc negatively affects the absorption of Fe by plants (Prasad et.al. 2016). Thus, with the increased availability of Zn in the soil, there was a disruption in absorption of Fe by orange trees hence Fe concentration decreased in the soil at both the Flatwoods and Ridge sites.

FRUIT YIELD. Generally, fruit yield increased at higher fertilization rates due to increased nutrient availability. Potassium availability increased with increased fertilization rate, thus enhancing functions such as fruit formation. These results are consistent with studies by Koo (1962), which showed an increase in fruit yield with increased K content while a decrease in yield was observed when K fertilizer was deficient in the treatments though this was done several years before the advent of HLB.

Conclusions

Nutrient availability for HLB-affected *Citrus sinensis* 'Valencia' orange trees is affected by interactions of nutrients in the soil. An increase in K concentration results in a decrease in Ca concentration while an increase in Zn concentration reduces Fe concentration in the soil for tree uptake. Optimum nutrient concentrations for K, Ca, Zn, and Fe in plant tissues of 1.9 ± 0.1 %, $4.0 \pm 0.13\%$, 48.7 ± 4.8 ppm and 45.5 ± 2.5 ppm respectively, are

 $^{^{}y}$ Means \pm SD followed by different lowercase letters are significantly different at P < 0.05 by Tukey's HSD test.

^{*}Standard fertilization + 1× macronutrient (MA) + 1× micronutrient (MI) (soil applied).

[&]quot;Standard fertilization + $1 \times MA + 2 \times MI$ (soil applied).

 $^{^{}v}$ Standard fertilization + 1× MA + 4× MI (soil applied).

[&]quot;Standard fertilization + $2 \times MA + 1 \times MI$ (soil applied).

^{&#}x27;Standard fertilization + 2× MA + 2× MI (soil applied).

 $^{^{\}circ}$ Standard fertilization + 2× MA + 4× MI (soil applied).

[&]quot;Standard fertilization + $1 \times MA + 2 \times MI$ (soil applied).

 $^{^{}v}$ Standard fertilization + 1× MA + 4× MI (soil applied).

^uStandard fertilization + $2 \times MA + 1 \times MI$ (soil applied).

 $^{^{}t}$ Standard fertilization + 2× MA + 2× MI (soil applied).

 $^{^{}s}$ Standard fertilization + 2× MA + 4× MI (soil applied).

suggested. For soil nutrient content, optimum nutrient concentrations of $83 \pm 36 \text{ mg} \cdot \text{kg}^{-1}$, $38 \pm 23 \text{ mg} \cdot \text{kg}^{-1}$ and $147 \pm 26 \text{ mg} \cdot \text{kg}^{-1}$ are suggested for Ca, Zn, and Fe, respectively. Higher fertilizer application rates increase nutrient availability, particularly K, which subsequently results in increased yield of HLB-affected orange trees.

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