

Nutrient Management Impacts on HLB-affected ‘Valencia’ Citrus Tree Growth, Fruit Yield, and Postharvest Fruit Quality

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Abstract. Since the first occurrence of Huanglongbing (HLB) in the Florida commercial citrus industry in 2004, fruit yield and yield components of HLB-affected citrus have declined in endemically affected citrus tree groves. Optimal fertilization is thus critical for improving tree performance because nutrients are vital for tree growth and development, and play a significant role in tree disease resistance against various biotic and abiotic stresses. The objective of the current study was to determine whether leaf nutrient concentration, tree growth, yield, and postharvest quality of HLB-affected citrus trees were improved by the split application of nutrients. The four micronutrient application rates were used as fixed factors and the three nitrogen (N) rates were used as random factors for leaf nutrient analyses, tree growth, fruit yield, and postharvest analyses. Significant leaf manganese (Mn) and zinc (Zn) concentrations were detected when trees received foliar and soil-applied micronutrients regardless of the N rates. There was a strong regression analysis of leaf Mn and Zn nutrient concentration and nutrient rates with R^2 : 0.61 and 0.59, respectively. As a result, a significant leaf area index associated with foliar and soil-applied micronutrient rates had a positive correlation with leaf area index and soil pH with R^2 : 0.58 and 0.63 during the spring and summer seasons, respectively. Trees that received a moderate ($224 \text{ kg}\cdot\text{ha}^{-1}$) N rate showed the least fruit decay percentage and total soluble solids (TSS) of 8% more than the lowest ($168 \text{ kg}\cdot\text{ha}^{-1}$) and highest ($280 \text{ kg}\cdot\text{ha}^{-1}$) N rates, even though fruit yield variations were barely detected as these micronutrients promoted vegetative growth. Moreover, the TSS to titratable acidity (TA) ratio of foliar and soil-applied micronutrient-treated trees showed 2% and 7% greater values than the foliar-only treated and control trees, respectively. Although micronutrients exacerbated stem-end rind breakdown (SERB), these nutrients significantly improved fruit storage when the fruits were stored for extended periods (8–11 weeks). Thus, moderate N rate, foliar (1×), and soil-applied (1×) micronutrient treatments improved tree growth, fruit postharvest, and fruit storage characteristics.

Nutrients are vital for tree growth, and development, and play a significant role in tree disease resistance against various biotic and

abiotic stresses (Dordas 2008; Uthman et al. 2022). The annual fertilizer rate, placement, sources, and timing of application (known as the 4R concept) contribute a fundamental role in maximizing nutrient use efficiency and minimizing nutrient leaching beyond the root zone (Morgan et al. 2006; Paramasivam et al. 2000, 2010). Because HLB (citrus greening) severely affects root health, inflicting 30% to 50% root loss early in disease development and greater than 70% root loss as

canopy decline begins, spoon-feeding citrus trees with optimal nutrient combinations should be a routine activity (Ghimire et al. 2020; Graham et al. 2013). HLB has spread in commercial citrus groves throughout the world except under intensive and restricted vector management systems (Gottwald et al. 2012). Since the first occurrence of HLB in the Florida commercial citrus industry in 2004, the relative decline in sweet orange acreage has been recorded at 42% (USDA 2022). Recently, researchers have vetted several mechanisms to boost yield and yield components of HLB-affected citrus in endemically affected citrus tree groves (Gottwald et al. 2012; Morgan et al. 2016; Zambon et al. 2019). Enhanced nutritional programs with or without vector control pesticides (Gottwald et al. 2012; Obreza and Schumann 2010; Phuyal et al. 2020; Stansly et al. 2014), the use of tolerant rootstocks and scions (Albrecht and Bowman 2019; Phuyal et al. 2020), and the use of antibiotics, hormones, and thermotherapy (Aubert 2008; Graham et al. 2020; Li et al. 2019) are among the recent adaptive mechanisms implemented to abate the severity of HLB-affected citrus trees.

Once citrus trees are infected with the HLB causal pathogen *Candidatus* Liberibacter asiaticus (CLAs), the tree experiences fibrous root decline (Hamido and Morgan 2020; Kumar et al. 2018; Wu et al. 2018), a nutrient deficiency, reduced tree growth (Uthman et al. 2020; Morgan et al. 2016), and shows symptoms of blotchy mottle on leaves, Zn deficiency (Cimò et al. 2013; Etxeberria et al. 2009; Gottwald et al. 2012), upper canopy twig dieback, veinal chlorosis, and foliar and fruit drop (Gottwald et al. 2012; Morgan et al. 2016). Mn is a crucial element for plant growth and facilitates several physiological processes such as photosynthesis and enzyme antioxidant-cofactor (Millaleo et al. 2010). Previous research indicated that Mn accumulation in tissues showed increment with time as the supply increased (Millaleo et al. 2010; Xue et al. 2004). Zn is also an essential element that interferes with membrane integrity, protein and hormone synthesis and activity, photosynthesis, lipid metabolism, gene expression, and plant defense mechanisms against stress (Morales-Payan 2022; Uthman et al. 2020). Research indicates that foliar Zn application increased vegetative growth in ‘Kinnow’ mandarin (Razzaq et al. 2013), Valencia oranges (Atta et al. 2021a), and ‘Improved Meyer’ lemon flower abundance, fruit weight, fruit yield, and quality in sweet orange (Morales-Payan 2022). In recent years, nutrition therapy has been used to reduce the severity of HLB symptoms (Morgan et al. 2016; Stansly et al. 2014; Zambon et al. 2019). Controlled release fertilizers (Esteves et al. 2021; Phuyal et al. 2020), split application of essential nutrients (Atta et al. 2021a; Esteves et al. 2021), enhanced nutritional programs (Gottwald et al. 2012; Stansly et al. 2014), and foliar and soil-applied of nutrients (Morgan et al. 2016; Uthman et al. 2020) had been effective in reversing the decline in

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anatomic, morphologic, and physiological anomalies associated with HLB disease.

Since the occurrence of HLB in Florida in 2004, the rate, timing, type, and placement of essential nutrients have been the topics of studies for better growth and yield of HLB-affected sweet oranges (Esteves et al. 2021; Morgan et al. 2016; Zambon et al. 2019). These nutrient application factors are included in the 4R concepts of the right rate, right time, right material, and right location. A combination of macronutrients and micronutrients had rigorously been under study in recent years in HLB-affected citrus groves. The effect of N, calcium (Ca), magnesium (Mg) (Atta et al. 2020b; Esteves et al. 2021; Phuyal et al. 2020), Mn, Zn, and boron (B) (Atta et al. 2021b; Hippler et al. 2015; Morgan et al. 2016; Uthman et al. 2020, 2022; Zambon et al. 2019) on HLB-affected citrus has been studied. Thus, two hypotheses were formulated: a) recurrent fertilization of the foliar coupled with the soil-applied application of essential nutrients Mn, Zn, and B improves leaf nutrient concentration and tree growth, and b) foliar and soil-applied essential nutrients also improve fruit yield, fruit drop, and juice content and quality of HLB-affected citrus trees. Therefore, the objective of the current study was to determine whether leaf nutrient concentration, tree growth, fruit drop, fruit yield, and postharvest quality of HLB-affected citrus trees were improved by the split application of varying fertilization rates, placements, and frequencies.

Materials and Methods

Site background, experimental setup, and treatments. The current study was conducted at the University of Florida, Southwest Florida Research and Education Center, Institute of Food and Agricultural Sciences (IFAS), located at Immokalee, FL (26.42° N, 81.43° W). Sweet orange trees (*Citrus × sinensis* L. Osbeck cv. Valencia late) budded on Swingle citrumelo (*Citrus × paradisi* Macf. × *Citrus trifoliata* L. Raf.) rootstocks that were planted in Apr 2006. The trees were planted in two-row north-south beds at an average tree density of 444 trees/ha. The trees were planted on Immokalee fine sand classified as sandy, siliceous, hyperthermic Arenic Alaquods (Kadyampakeni et al. 2014b). Leaf sample analysis indicated an HLB level of the bacteria CLas using real-time quantitative polymerase chain reaction (qPCR) with a cycle threshold value of 24.7 ± 0.2 (Atta et al. 2021b).

The experiment was designed as a split-plot design in which the treatments were assigned at random. In the main plot, the trees received one of the three N rates 168, 224, or 280 kg/ha/year, where in the sub-plots the micronutrients were as follows: control 0× (0×), foliar only 1× (1×), foliar and soil-applied 1× of each (2×), or foliar at 1× and soil-applied 2× (3×) treatments were applied. The treatment 1× corresponded to 9 kg·ha⁻¹ Mn and Zn oxides and 2.3 kg·ha⁻¹ of B nutrients as earlier recommended by the University of Florida/IFAS for citrus nutrition (Obreza and

Morgan 2008). The K fertilizer was applied equally at the same rate of 140 kg·ha⁻¹ per year. The N was fertigated biweekly from February to November in 20 split applications per year. The micronutrient treatments were applied three times a year at the beginning of the spring (March), at the middle of the summer (June), and at the end of summer (September) leaf flush (Atta et al. 2021b; Jenkins et al. 2015). The foliar treatments were applied using a truck-mounted sprayer motor pump (Hypro corporation, New Brighton, MN) connected to a sprayer GunJe (Spraying Systems Co., Glendale Heights, IL) with a pressure chamber capacity of 5.5 × 10⁶ Pa [AA(B) 43L-AL4 (Spraying Systems Co., Glendale Heights, IL)]. A stopwatch for the uniformity of the treatments estimated the volume of the solution applied to each experimental unit (Atta et al. 2020a).

Leaf sampling and analysis. Mature 20 to 25 leaf samples per tree (n = 12) were collected from nonfruiting branches in the spring (February or March) and summer (August or September) seasons of 2019 and 2020 (Morgan et al. 2016; Obreza and Morgan 2008). The leaf samples were washed in a weak (0.1% to 0.3%) Micro90 detergent solution (Micro90 International Products Cooperation, Burlington, NJ, USA), rinsed in reverse osmosis water, and subsequently with deionized water to remove grime adhering to the leaf surface. Once the leaf samples were oven-dried at 65 °C for 72 h and reached a constant weight, they were ground to pass through a 40-mesh screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) (Morgan et al. 2006; Uthman et al. 2020). The fine-powder oven-dried leaf tissue sample of 0.5 ± 0.005 g was weighed, placed in 40-mL glass tubes, and ashed at 500 °C for 16 h. The furnace door was gently opened at a temperature of less than 200 °C to avoid oxidation of the ashy leaf samples by the rapid inflow of air.

The ashed samples were equilibrated with 15 mL 0.5 M HCl at room temperature for 30 min using an adjustable macro-pipette to digest the ashes. The sample solution was decanted into 25-mL glass tubes and kept at < 4 °C pending analysis. The sample solution was analyzed with inductively coupled plasma optical emission spectroscopy (Spectro Ciros CCD, Fitzburg, MA, USA) (Munter et al. 1984). Leaf N was also estimated from the same ground samples using the NA2500 carbon (C)/N analyzer (Hiden Analytical Ltd., Warrington, UK). Meanwhile, the leaf nutrient concentration was compared with the critical nutrition concentration that had previously been established from years of experimentation (Obreza and Morgan 2008).

Tree leaf area index and canopy volume. The measurement of leaf area index (LAI) was estimated by the amount of solar radiation transmitted through the tree canopy. The LAI was estimated by the solar radiative transfer principle by a SunScan canopy sensor system (Dynamax Inc., Houston, TX, USA) at noon (1130 to 1330 HR). The accuracy of the data were assumed when the solar zenith angle was within < ±10° (±0.1) (Atta

et al. 2020b). The average of four readings per tree (n = 12 trees) data were collected in the direction of east, west, north, and south from the tree trunks. Tree canopy volume (CV) was estimated by measuring the average canopy width in the east-west and north-south directions, and canopy height using a leveling staff. The CV was measured twice per year in the spring (February or March) of the experiment and every 6 mo afterward (August or September) from 2019 to 2021. The CV volume was calculated using the formula for a prolate spheroid (Kadyampakeni et al. 2016):

$$CV = \frac{4}{3} \times \pi \times r^2 \times \left(\frac{h}{2}\right) \quad [1]$$

where CV (m³) = tree canopy volume, r = average canopy radius (m), and h = canopy height (m).

Fruit postharvest quality. Fruit yields associated with previous nutrient studies of HLB affected have been documented (Morgan et al. 2016; Uthman et al. 2020; Zambon et al. 2019). However, differences in fruit quality have not. Fruit yield per tree was estimated from (n = 12) trees harvested in March of 2019–20 to 2021–22 seasons. Data on preharvest fruit drops were also collected 3 mo before and immediately after the commercial fruit harvest. One characteristic of HLB-affected trees is an increased loss of fruit before harvest (fruit drop), resulting in reduced fruit yield (Albrigo and Stover 2015; Atta et al. 2021b; Gottwald et al. 2012; Graham et al. 2013). The number of fruit drops per tree was converted to fruit drop weight by multiplying by the average weight of 30 fruits to obtain the average fruit drop weight per tree (kg/tree) (Gottwald et al. 2012; Paramasivam et al. 2000). Sixty fruit samples were collected to get data for juice content, TSS, TA, the ratio of TSS and TA, and health status (decay and SERB) levels after 4 and 8 weeks of storage. Fruits were evaluated after 11 weeks of storage in 2020 (COVID delayed), the fourth and eighth weeks of storage in 2021, and the third and sixth weeks of storage in 2022. No postharvest fungicides were applied. Fruit juice content was determined using 20 fruits per replicate, weighed, and extracted using a mechanical juice extractor (model 2702; Brown International Corp., Covina, CA, USA). The juice content was weighed to determine the juice percentage (Gottwald et al. 2012) as:

Juice percentage =

$$\frac{\text{Total weight of juice (g)}}{\text{Total weight of fruit (g)}} \times 100 \quad [2]$$

The TSS was estimated using a digital refractometer (ATAGO, PAL-1 BLT/i, Atago, Japan), at room temperature, and expressed as °Brix. Titrable acidity (TA) of fruit juice was processed by the method given by Hortwitz (1960) and was expressed as percent anhydrous citric acid. The TSS:TA ratio was calculated by dividing the TSS by the corresponding TA value. Both fruit decay and

SERB were examined at regular intervals and the experiment was halted when the fruit developed a substantial amount of decay or SERB (about 20% to 50%). Decayed fruits were removed from the experiment at each time of inspection while SERB was maintained until the end of the experiment. The presence of any SERB or decay symptoms caused the fruit to be scored in the appropriate category. Cumulative percentages of decayed or SERB fruit were reported in the fourth and eighth weeks at 4.4 °C (Ritenour et al. 2004). The SERB is featured as the disintegration of rind tissues around the stem end of a citrus fruit whereby the affected spot is manifested by an irregular shape and becomes dark and sunken, which is associated with the physiological disorder that poses economic loss (Rezaee et al. 2020; Ritenour et al. 2004). The SERB percentage was calculated according to Rezaee et al. (2020):

SERB (%) =

$$\frac{\text{number of damaged fruits in each replication}}{\text{total fruits of each replication}} \quad [3]$$

Statistics and data analysis. The four micronutrient treatment rates were used as fixed factors and the three N rates were used as random factors for leaf nutrient analyses, tree CV, LAI, and fruit yield and postharvest quality parameters. Repeated-measures analysis PROC GLM Model procedures from SAS 9.4 (version 14.1; SAS Institute, Cary, NC, USA) were used for data analyses. When data failed to meet the basic statistical assumptions, the log transformation was used to test for normality, linearity, independent errors, and homoscedasticity. For treatments with F-tests showing a statistical difference ($P \leq 0.05$), the Tukey-Kramer honestly significant difference multiple range test was used to compare the means. The logarithmic regression of micronutrient rates and leaf nutrient concentrations and the quadratic regression analysis of LAI and soil pH were tested using Sigma Plot 14 (SigmaPlot 14; Systat Software, San Jose, CA, USA).

Result and Discussion

Leaf Mn concentration. With the fixed effect of the N rates, we investigated how the leaf Mn concentration was affected according to the micronutrient rates. During spring 2019, significantly greater leaf Mn concentration was detected when trees received foliar (1×) and soil-applied (1× or 2×) Mn fertilizer than only foliar (1×) or control treatments irrespective of the N rates (Fig. 1A and B). With the higher N rates (224 and 280 kg·ha⁻¹) a significantly greater leaf Mn concentration was detected only in the soil and foliar treatments during Spring 2020. Conversely, the leaf Mn concentration was significantly greater when trees received foliar Mn fertilizer than foliar only or control treatment trees regardless of the N rates in the summer season (Fig. 1C and D). The results from these two seasons indicated that the uptake of Mn increased with increasing N rates. In addition,

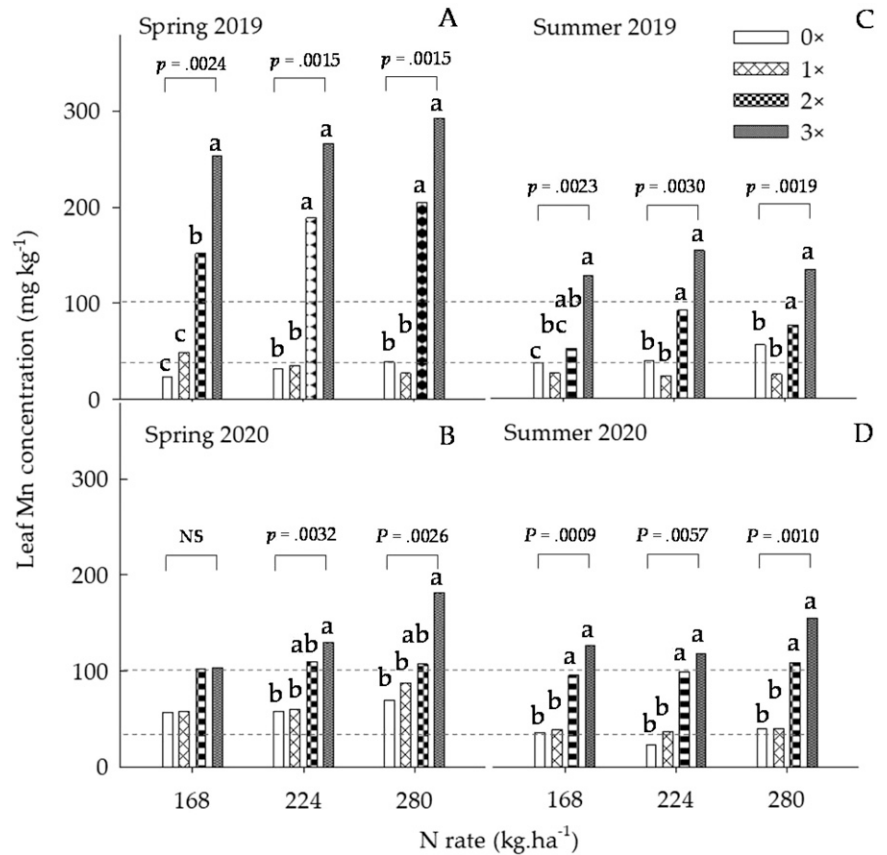


Fig. 1. Leaf manganese (Mn) concentration at three nitrogen (N) rates as affected by micronutrient rates: control (0×), foliar only 1× (1×), foliar and soil-applied 1× of each (2×), and foliar at 1× and soil-applied 2× (3×). Micronutrients Mn, zinc (Zn), and boron (B) (1× = 9 kg·ha⁻¹ per year of Mn and Zn each and 2.3 kg·ha⁻¹ per year of B). Horizontal dashed lines across the four panels indicate the optimum leaf concentration for Florida citrus tree nutrition.

the relatively low leaf Mn concentration during the summer as compared with the spring season was also an indication of the dilution effect because of the massive vegetative growth during the summer seasons. This indicated that adding an extra 1× to the soil resulted in excessive leaf Mn concentration. Furthermore, the relative increase in leaf Mn concentration under foliar 1× and soil-applied 2× (3×) treatments could not significantly increase as compared with foliar 1× and soil-applied 1× (2×) treatments. However, only foliar application cannot be a guarantee to maintain the leaf Mn concentration within the optimum leaf nutrient concentration. Previous research indicated that leaf Mn concentration had a value of 3.8-fold, 4.4-fold, and 6.8-fold greater than the control trees attributed to the foliar only 1× (1×), foliar 1× and soil-applied 1× (2×), and foliar 1× and soil-applied 2× (3×) treatments, respectively (Atta et al. 2021b). Similarly, studies indicated that about 80% root Mn concentration and 58% lesser leaf tissue concentration were detected in HLB-affected than HLB-free citrus trees (Hippler et al. 2015; Zambon et al. 2019).

Leaf Zn concentration. The leaf Zn concentration was significantly greater under foliar and soil-applied treatments in the spring seasons than in the untreated control trees (Fig. 2A and B). Only foliar (1×) and foliar

1× and soil-applied 1× (2×) treatments showed no significant variation under 168 kg·ha⁻¹ and 224 kg·ha⁻¹ N rates. This indicated that the uptake of Zn and accumulation on the leaf tissues increased with N rates as the highest N rate showed the highest leaf Zn concentration under both foliar and soil-applied treatments. It was imperative that the magnitude of the leaf Zn concentration increased in summer and showed the highest concentration on the treated than control trees regardless of the N rates (Fig. 2C and D). However, there was no significant variation in leaf Zn concentration among the treated trees. Thus, the foliar-only application could satisfy the yearly Zn requirements. Soil immobilization, leaching of soil Zn, crop removal in the summer, spring fruit harvest, and defoliation of leaves, branches, and twigs in the fall could be the reason for the drop in leaf Zn concentration in the spring seasons (Atta et al. 2021b). The drop in spring and increase in the summer season of leaf Zn concentration is an indication of higher translocation of leaf Zn bioaccumulating from the source to the new growth points (Atta et al. 2021b; Zambon et al. 2019). In additionally, high soil Zn immobilization and root damage associated with HLB-induced conditions were also other indicators of reduced Zn mobility from the soil to the leaf (Fu et al. 2016).

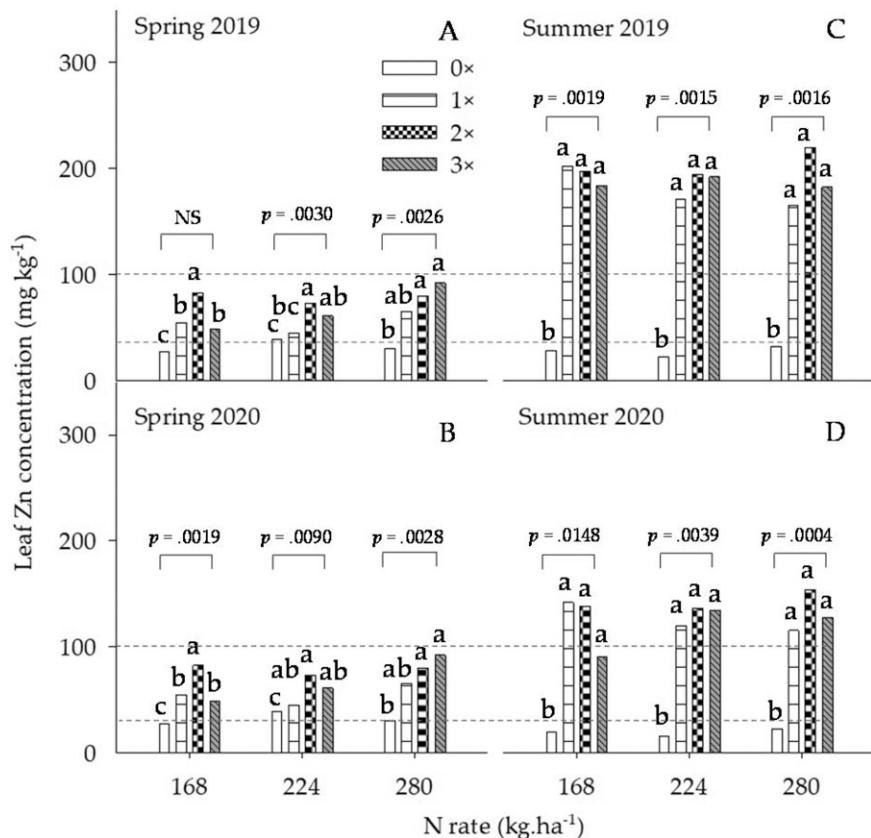


Fig. 2. Leaf zinc (Zn) concentration at three nitrogen (N) rates as affected by micronutrient rates: control (0×), foliar only 1× (1×), foliar and soil-applied 1× of each (2×), and foliar at 1× and soil-applied 2× (3×). Micronutrients manganese (Mn), Zn, and B rates (1× = 9 kg·ha⁻¹ per year of Mn and Zn each and 2.3 kg·ha⁻¹ per year of B). Horizontal dashed lines across the four panels indicate the optimum leaf concentration for Florida citrus tree nutrition.

There were significant logarithmic relationships ($R^2 = 0.61$ and $R^2 = 0.59$) between leaf Mn and Zn concentrations in response to the micronutrient rates, respectively (Fig. 3A and B). Leaf Mn concentration remained below the optimum nutrient ranges indicating

that HLB-affected trees required supplementary nutrients. When trees received only foliar applications, the leaf Mn remained around the minimum line of the optimum nutrient ranges for Florida's citrus nutrition. These values may not be a guarantee of future

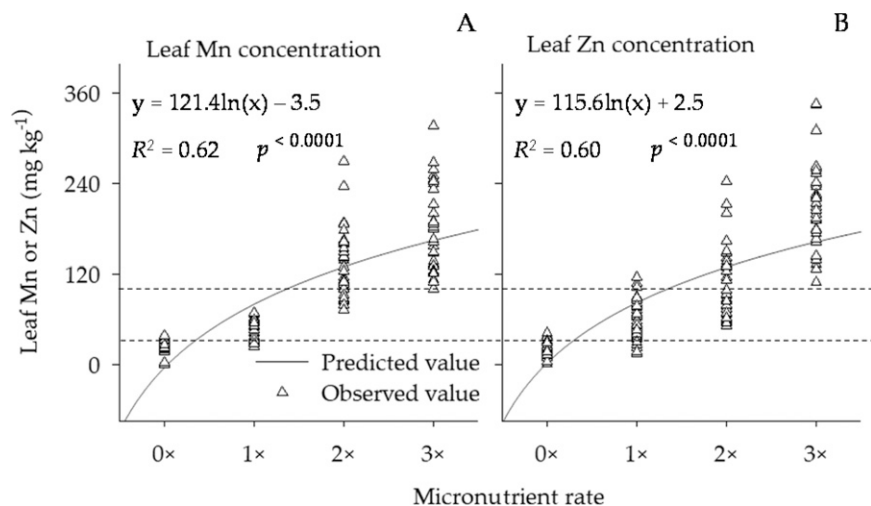


Fig. 3. The logarithmic regression analysis of leaf manganese (Mn) (A) and zinc (Zn) (B) nutrient concentration and micronutrient rates. Micronutrient rates: control (0×), foliar only 1× (1×), foliar and soil-applied 1× of each (2×), and foliar at 1× and soil-applied 2× (3×) treatment of which (1× = 9 kg·ha⁻¹ per year of Mn and Zn each and 2.3 kg·ha⁻¹ per year of boron). Horizontal dashed lines across the four panels indicate the optimum leaf concentration for Florida citrus tree nutrition.

biological growth because trees may experience heavy leaf drops, crop removal, and fruit harvest season. Thus, foliar 1× and soil-applied 1× (2×) treatments could maintain the optimum crop nutrient requirements; however, foliar 1× and soil-applied 2× (3×) treatment had exceeded the optimum range of leaf Mn or Zn concentrations for the nutrition of Florida citrus trees. It is noted that HLB-affected trees are deficient in leaf Mn and Zn concentrations supporting previous studies. Therefore, only foliar Zn nutrient application demonstrated that the trees could fulfill the yearly crop requirement supporting the above discussions. Foliar and simultaneous soil-applied Zn resulted in higher than leaf Zn concentrations.

LAI and CV. The 3-year study indicated that the variation in LAI was detected at the higher N rates than the lowest N rate (Table 1). Meanwhile, the LAI significantly increased with increasing micronutrient rates but showed a significantly lower LAI with the highest micronutrient rate. Micronutrient-related variations in LAI were significantly prevalent when trees received higher N rates. On average, LAI showed an increase of 1.10-fold, 1.00-fold, and 0.83-fold pertained to the foliar only 1× (1×), foliar 1× and soil-applied 1× (2×), and foliar 1× and soil-applied 2× (3×) treatments as compared with the control trees, respectively. Like the LAI, the micronutrients showed a significant effect on tree CV (Table 2). There was an increase of 1.06-fold, 0.99-fold, and 0.78-fold in response to the foliar only 1× (1×), foliar 1× and soil-applied 1× (2×), and foliar 1× and soil-applied 2× (3×) treatments as compared with the control trees, respectively. Persistently, the trees under the highest micronutrient rates showed a decrease in LAI and tree CV.

In the previous study on S-encapsulated Mn and Zn treatments, a significantly lower LAI, CV, and fine root length density were detected even if there were significant leaf Mn and Zn concentrations (Atta et al. 2020a). In the previous study, soil pH declined with increased soil acidity caused by the S encapsulation and could have resulted in soil Al³⁺ dissociation and release into the soil solution that might eventually hinder root growth and lead to a decline in LAI (Atta et al. 2020a; Brunner and Sperisen 2013). In the meantime, there were significant quadratic relationships ($R^2 = 0.58$ and $R^2 = 0.63$) between soil acidity and LAI during the spring and summer seasons, respectively (Fig. 4A and B). Higher LAI and activity of elemental S resulted in lower soil pH during the summer season. Accordingly, the soil pH was higher during the spring season, but with lower LAI values as opposed to the lower soil pH and higher LAI during the summer seasons. As above-ground growth is associated with root growth, extended root exposure to elevated H⁺ activity in the tree root zone could negatively affect the veracity of root plasma membrane permeability, disrupting the electrochemical balance, and ultimately affecting plant nutrient uptake and growth (Brunner and Sperisen 2013).

Fruit yield and postharvest fruit quality. With increasing N rate and micronutrients, fruit

Table 1. Mean of leaf area index of Huanglongbing (HLB)-affected ‘Valencia’ citrus trees (n = 12 trees) as affected by essential nutrients.

	Leaf area index								
	2019			2020			2021		
	Nitrogen rates (kg·ha ⁻¹)								
Micro ⁱ	168	224	280	168	224	280	168	224	280
0×	3.1 ± 0.8 ⁱⁱ	3.9 ± 0.2 a	3.5 ± 0.8 ab	3.7 ± 0.2	3.7 ± 0.2 b	3.2 ± 0.2 c	3.0 ± 0.3	3.1 ± 0.3 a	3.3 ± 0.3 a
1×	4.0 ± 0.3	4.1 ± 0.4 a	3.7 ± 0.7 ab	4.4 ± 0.4	4.5 ± 0.4 a	4.0 ± 0.4 ab	3.0 ± 0.4	3.0 ± 0.3 ab	3.3 ± 0.4 ab
2×	4.0 ± 0.2	4.0 ± 0.2 a	4.1 ± 0.5 a	4.1 ± 0.0	4.2 ± 0.4 a	4.0 ± 0.4 a	3.2 ± 0.2	3.0 ± 0.1 ab	3.1 ± 0.4 ab
3×	2.6 ± 0.3	2.3 ± 0.3 b	2.9 ± 0.5 b	3.6 ± 0.6	3.4 ± 0.5 b	3.4 ± 0.5 c	3.0 ± 0.3	2.7 ± 0.3 b	2.3 ± 0.4 b
P value	0.374	0.0001	0.0101	0.228	0.0082	0.0086	0.1276	0.0361	0.0211

ⁱ Micro: control (0×), foliar 1× (1×), foliar and soil-applied each 1× (2×), and foliar 1× and soil-applied 2× (3×), (1× = 9 kg·ha⁻¹ per year of manganese and zinc each and 2.3 kg·ha⁻¹ per year of boron).

ⁱⁱ Values on the same column followed by the same letters are not significantly different based on Tukey-Kramer honestly significant difference multiple range test at $P \leq 0.05$.

Table 2. Tree canopy volume of Huanglongbing (HLB)-affected ‘Valencia’ citrus trees (n = 12 trees) as affected by essential nutrients in Immokalee, FL, during the spring and summer seasons of 2019–21.

	Canopy volume (m ³)								
	2019			2020			2021		
	Nitrogen rate (kg·ha ⁻¹)								
Micro ⁱ	168	224	280	168	224	280	168	224	280
0×	18.2 ± 2.2 ⁱⁱ	19.9 ± 0.2	18.2 ± 2.2 b	22.0 ± 1.2	22.4 ± 2.4	20.4 ± 1.2 b	20.3 ± 1.3	21.1 ± 1.4	19.7 ± 1.3 b
1×	21.4 ± 1.6	21.7 ± 0.1	17.5 ± 2.9 b	24.3 ± 1.6	21.5 ± 4.6	21.5 ± 1.3 b	24.9 ± 2.0	23.5 ± 4.0	20.1 ± 0.9 b
2×	22.5 ± 1.8	20.6 ± 0.2	24.9 ± 0.4 a	24.5 ± 3.0	22.8 ± 1.9	26.2 ± 1.3 a	24.7 ± 1.2	21.5 ± 1.2	25.9 ± 1.6 a
3×	17.9 ± 1.8	21.1 ± 0.2	19.7 ± 0.9 b	22.5 ± 1.7	25.9 ± 2.1	21.2 ± 1.7 b	24.9 ± 3.1	24.3 ± 2.9	19.5 ± 2.1 b
P value	0.199	0.905	<0.0001	0.887	0.602	0.0064	0.703	0.384	0.0008

ⁱ Micro: control (0×), foliar 1× (1×), foliar and soil-applied each 1× (2×), and foliar 1× and soil-applied 2× (3×), (1× = 9 kg·ha⁻¹ per year of manganese and zinc each and 2.3 kg·ha⁻¹ per year of boron).

ⁱⁱ Values on the same column followed by the same letters are not significantly different based on Tukey-Kramer honestly significant difference multiple range test at $P \leq 0.05$.

yield showed an increasing trend on the treated trees compared with control trees (Table 3). Because trees first allocate photosynthetic products for vegetative growth, increasing LAI and CV as discussed earlier might have a competitive effect on fruit yield. As a result, significant fruit yield was barely detected in any of the treated trees. The fruit drop was more pronounced in the treated trees than in the control trees. Thus, results in the current

study indicated that the split application of nutrients resulted in greater fruit production but had increased fruit drop ranging from 13% to 37% resulting in nearly equal harvestable yields. Previous fruit drop studies by USDA estimated 18% and 23% for early and midseason sweet orange cultivars (Hamlin, Midsweet, and Pineapple) and 22% and 31% ‘Valencia’ sweet oranges for the 2012–13 and 2013–14 harvest seasons, respectively

(Albrigo and Stover 2015). However, the pre-harvest fruit drop of HLB-affected citrus trees ranged from 31% to 74% (Albrigo and Stover 2015; Gottwald et al. 2012). This result suggested that most of the fruit drop was more prevalent in the preharvest than during the fruit harvest due to mechanical shaking by fruit pickers.

There was no significant effect of macronutrients and micronutrients on the content of fruit juice. Yet, the fruit juice content was not below the average recorded in previous studies (Gasque et al. 2016; Quaggio et al. 2006). The TSS was highest when trees received a moderate N rate (224 kg·ha⁻¹) and with the highest micronutrient foliar and soil-applied nutrients (Table 4). The 224 kg·ha⁻¹ N rate had 8% more TSS than the lowest (168 kg·ha⁻¹) and highest (280 kg·ha⁻¹) rates. Similarly, TSS was reported higher with 185 kg·ha⁻¹ N than 100 kg·ha⁻¹ (Quaggio et al. 2006). The TA of the control trees was 9% and 7% greater than the moderate (224 kg·ha⁻¹) and the highest (280 kg·ha⁻¹) N rates, respectively.

Meanwhile, the TSS:TA ratio of foliar (1×) and soil-applied (1×) micronutrient treatments was 2% and 7% greater than the foliar-only (1×) treatments and control trees, respectively. Trees had significantly higher TA under the control and the highest micronutrient rates indicating 5% and 12% less TA under foliar (1×) and soil-applied (1×) and foliar-only (1×) treatments, respectively. This indicated that the increase in vegetative

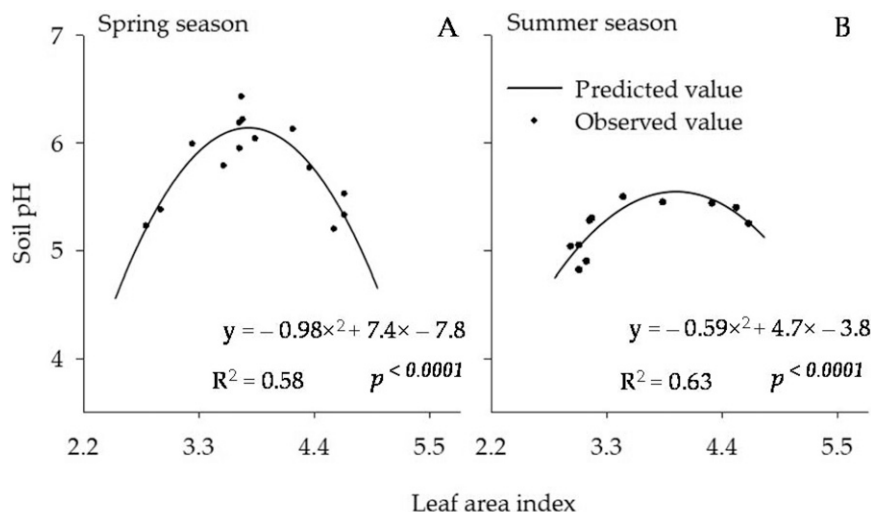


Fig. 4. The quadratic regression analysis of leaf area index and soil pH of Huanglongbing (HLB)-affected ‘Valencia’ citrus trees in Immokalee, FL, during the spring (A) and summer (B) seasons of 2020–21.

Table 3. Effect of essential nutrients on fruit yield and fruit drop of Huanglongbing (HLB)-affected citrus trees.

Micro ⁱ	2019/2020			2020/2021			2021/2022		
	Fruit yield (kg/tree)								
	Nitrogen rate (kg·ha ⁻¹ per year)								
	168	224	280	168	224	280	168	224	280
0×	74.5 ± 23 ⁱⁱ	82.9 ± 3	59.1 ± 23	46.7 ± 4	54.2 ± 11	59.2 ± 4	25.8 ± 7	41.5 ± 2	29.6 ± 7
1×	71.7 ± 4	71.1 ± 16	73.5 ± 19	55.2 ± 7	51.4 ± 7	50.2 ± 4	35.8 ± 2	35.6 ± 8	36.8 ± 7
2×	64.0 ± 17	83.2 ± 4	93.1 ± 12	61.7 ± 11	48.2 ± 16	51.6 ± 5	32.0 ± 9	41.6 ± 2	46.6 ± 3
3×	62.8 ± 18	78.1 ± 16	78.0 ± 5	49.1 ± 10	53.8 ± 9	51.8 ± 7	31.4 ± 9	39.0 ± 8	39.0 ± 2
	Fruit drop (kg/tree)								
0×	9.9 ± 2	18.5 ± 1	12.2 ± 2	14.4 ± 4	16.0 ± 3	20.3 ± 4	10.7 ± 3	11.7 ± 1	10.3 ± 3
1×	10.9 ± 2	16.8 ± 2	14.6 ± 2	14.2 ± 4	19.1 ± 3	11.7 ± 4	8.8 ± 2	9.5 ± 1	7.0 ± 3
2×	16.0 ± 3	20.8 ± 6	12.2 ± 3	15.9 ± 4	16.3 ± 4	17.4 ± 5	11.5 ± 1	9.6 ± 0	7.2 ± 2
3×	10.6 ± 3	14.2 ± 3	17.5 ± 3	16.9 ± 5	11.0 ± 2	14.2 ± 4	10.1 ± 1	6.3 ± 1	5.3 ± 3

ⁱ Micros: control (0×), foliar 1× (1×), foliar and soil-applied each 1× (2×), and foliar 1× and soil-applied 2× (3×), (1× = 9 kg·ha⁻¹ per year of manganese and zinc each and 2.3 kg·ha⁻¹ per year of boron).

ⁱⁱ Values are the mean (n = 12 trees) ±SEM of 3 years of studies, 2019–21.

growth associated with the micronutrients could result in decreasing TA and increased TSS. The TSS, TA, and TSS:TA ratio essentially determine the sweetness and flavor that are desirable traits of citrus fruit for fruit quality control and assurance (Ncama et al. 2017). Previous studies have found that HLB can reduce sweetness (soluble solids content) while increasing acidity (Dala-Paula et al. 2019; Ncama et al. 2017). Hence, applying micronutrient fertilizer may have increased

the quality of the fruit associated with the aforementioned fruit quality parameters as compared with the control trees.

A significant SERB was detected on foliar (1×) and soil-applied (1×) treatments of 22% greater than the control trees. Previous research revealed that nutritional disproportions involving N and potassium affect fruits to SERB followed by decay (Porat et al. 2004; Ritenour et al. 2004). Fruit decay percentage was the least when trees received moderate N

rates of 12% and 19% as compared with the control and the highest N rates, respectively. Previous studies indicated that the SERB index had negative correlations with total sugars and decreased sugar content (Rezaee et al. 2020). Even though the fruit health status of the control trees showed 12% healthier during the earliest storage weeks; late storage/shelf life (eighth week) of the foliar and soil-applied micronutrient treatments had 15% healthier than the control trees. These findings

Table 4. Effect of essential nutrients on fruit postharvest fruit quality of Huanglongbing (HLB)-affected citrus trees.

N rate ⁱⁱ	Juice Content (%)	TSS	TA	TSS/TA	After 4 wk of storage			After 8 wk of storage ⁱ		
					Healthy (%)	Decay (%)	SERB (%)	Healthy (%)	Decay (%)	SERB (%)
2019/2020 Harvest										
168	40.5	10.2	0.66	15.6 b ⁱⁱⁱ	– ^{iv}	–	–	22.5	11.4	66.1
224	38.1	10.8	0.64	17.1 ab	–	–	–	21.5	8.0	70.5
280	38.7	11.1	0.61	18.2 b	–	–	–	20.6	10.5	68.9
Micro ^v				<i>P</i> = 0.015						
0×	55.1 a	10.7	0.63 ab	16.9 ab	–	–	–	30.0 a	9.9	60.2 b
1×	33.9 ab	10.7	0.65 ab	16.6 ab	–	–	–	19.2 b	10.2	70.7 ab
2×	29.9 b	10.9	0.61 b	18.2 a	–	–	–	16.6 b	10.1	73.3 a
3×	37.6 b	10.5	0.66 a	16.1 b	–	–	–	20.4 ab	9.8	69.9 ab
N rate	<i>P</i> = 0.003		<i>P</i> = 0.028	<i>P</i> = 0.034				<i>P</i> = 0.005		<i>P</i> = 0.017
168	50.5	8.6 b	0.78	11.2 b	51.9	4.1	44.0	19.1	32.6 a	48.3
224	48.7	9.3 a	0.76	12.5 a	59.1	6.8	34.1	22.8	29.5 ab	47.8
280	50.8	8.6 b	0.77	11.3 b	64.5	3.2	32.3	27.4	24.6 b	48.0
Micro		<i>P</i> = 0.012			<i>P</i> = 0.008				<i>P</i> = 0.021	
0×	50.0	8.8 ab	0.74	12.1 a	59.9 ab	5.5	34.6 b	23.7 ab	29.6	46.76
1×	51.3	8.4 b	0.79	10.8 b	48.2 b	5.3	46.5 ab	14.9 b	33.3	51.8
2×	49.3	9.0 a	0.79	11.5 ab	62.2 a	4.6	33.2 a	27.2 ab	27.9	44.98
3×	49.5	9.0 a	0.75	12.4 a	63.3 a	3.4	33.0 ab	26.6 a	24.8	48.59
N rate		<i>P</i> = 0.001		<i>P</i> = 0.041	<i>P</i> = 0.004	2021/2022	<i>P</i> = 0.034	<i>P</i> = 0.040		
168	53.1	9.0	0.72 a	12.5	98.7	4.4	2.6	17.7	17.7	31.5
224	52.5	9.1	0.66 b	15.9	92.3	3.2	3.7	24.2	24.2	32.2
280	51.8	8.9	0.67 ab	12.7	98.4	3.3	2.6	23.7	23.7	35.5
Micro			<i>P</i> = 0.0136							
0×	53.3	8.9	0.68	13.1	98.3	3.2	2.5	39.6	39.6	36.3
1×	54.1	9.1	0.71	13.0	98.7	5.3	4.9	52.7	52.7	27.9
2×	50.9	9.2	0.67	14.1	97.9	3.7	2.6	42.0	42.0	33.4
3×	51.7	8.9	0.67	13.5	91.0	2.5	2.6	43.9	43.9	34.6

ⁱ Fruits were evaluated after 11 weeks of storage in 2020 (COVID-19 closures), fourth and eighth weeks of storage in 2021, and third and sixth weeks of storage in 2022.

ⁱⁱ Nitrogen rates 168, 224, and 280 kg·ha⁻¹ per year.

ⁱⁱⁱ Values on the same column followed by the same letters are not significantly different based on Tukey-Kramer honestly significant difference multiple range test at *p* ≤ 0.05.

^{iv} No data available due to COVID-19 shutdown.

^v Micronutrients: control (0×), foliar 1× (1×), foliar and soil-applied each 1× (2×), and foliar 1× and soil-applied 2× (3×), (1× = 9 kg·ha⁻¹ per year of manganese and zinc each and 2.3 kg·ha⁻¹ per year of boron).

TA = titratable acidity; TSS = total soluble solids; SERB = stem-end rind breakdown.

suggested that micronutrients affected the shelf life of fruits.

Conclusions

Significantly higher tree LAI was found in trees receiving the combination of 1× foliar and 1× ground applied micronutrients at the 224 and 280 kg·ha⁻¹ rates, whereas higher CV was measured when trees received foliar (1×) and soil-applied (1×) micronutrient rates at the high N rate of 280 kg·ha⁻¹ only. Leaf Mn and Zn concentrations also showed similar increments with increased N rates. Excessive foliar (1×) and 2× soil-applied micronutrients (3× treatment) had a detrimental effect on tree growth, postharvest fruit quality, and storage characteristics. Even though N rate and micronutrient applications did not affect fruit yield, trees that received a moderate (224 kg·ha⁻¹) N rate and 2× micronutrient treatments showed greater TSS and lower acidity in 1 of 3 years, which are key measurements of juice quality. Fruit from the highest N rate had the lowest fruit decay percentage at 8 weeks of storage and 2× micronutrient treatment increased SERB at 4 weeks of storage, indicating improved fresh fruit shelf life. Thus, a moderate N rate, and a combination of foliar (1×), and soil-applied (1×) micronutrient treatments improved tree growth, fruit postharvest, and storage characteristics.

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