Proc. Fla. State Hort. Soc. 131:58–64. 2018.

Effect of Soil and/or Foliar Applied Nutrients on Leaf Nutrient Accumulation and Water Uptake on Huanglongbing Affected 'Valencia' Citrus Trees

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Additional index words. split application, foliar application, water and nutrient uptake, citrus greening

Citrus production in Florida started to decline in 2005 due to citrus greening, Huanglongbing (HLB) disease. HLB affects citrus tree physiology and morphology, subsequently affecting nutrient and water uptake and utilization. The main objective of this study was to evaluate if soil and/or foliar applied nutrients affect leaf nutrient concentration and water uptake of HLB affected *Citrus sinensis* 'Valencia' trees. The experiment was set up in a split-split-plot design comprised of two rootstocks, three nitrogen (N) rates, and foliar and/or soil applied manganese (Mn), zinc (Zn), and boron (B) nutrition. Results indicated that persistent split N application for one year brought HLB affected N deficient citrus trees to the optimum range of critical nutrient concentration for Florida citrus nutrition (CCFCN) with lower N rate requirement. Leaf analysis indicated significantly higher Mn and Zn leaf concentration in trees that received either foliar only or foliar and soil applied micronutrients compared to the control (untreated). Leaf B concentrations were above the optimum range regardless of the season, year, and N treatment, so no conclusions regarding effect of application could be made. Water stress levels as measured by stem water potential were significantly higher for trees budded on Swingle than Volkameriana (Volk) rootstock. In Spring 2017, significantly higher water stress was noted with lower foliar applied rate compared with the highest soil and foliar micronutrient rates only on trees budded on Swingle rootstocks. The current study suggests that the foliar application of Mn and Zn was enough to satisfy tree nutrition demand with at least one or two soil-applied Mn and Zn treatments in addition to foliar application after massive vegetative loss caused by hurricane and recurrent leaching rain events.

Florida is ranked first in the United States followed by California for orange production accounting for 57% and 43%, respectively [United State Department of Agriculture (USDA, 2017a). Nevertheless, citrus production has been declining since 2005. The reasons for the decline include damage from Hurricane Irma, urban encroachment, canker (*Xanthomonas axonopodis*), and Huanglongbing (HLB, citrus greening) disease (Morgan et al., 2009a; Kadyampakeni et al., 2016; USDA, 2017b). The bacterium that is associated with HLB, *Candidatus* Liberibacter asiaticus, is introduced into a citrus tree by phloem-feeding psyllids (ACP, *Diaphorina citri*) causing reduced root density of 50% or more, which ultimately affects nutrient uptake, movement, assimilation, and utilization (Hamido et al., 2017; Kadyampakeni et al., 2014a; Spann and Schumann, 2009b). HLB affected citrus trees with reduced fibrous roots, develop HLB induced visual symptoms such as interveinal chlorosis of young leaves, trailed by blotchy mottling of older leaves, and later in the season results in over accumulation of starch that distort the grana in chloroplast (Etxeberria et al., 2009; Morgan et al., 2016). Research results indicate that water uptake by HLB affected citrus trees was about 18 to 29% lower than healthy citrus trees (Morgan et al., 2016). Citrus growers in Florida have been using foliar spray of microelements [manganese (Mn), zinc (Zn), and boron (B)] as a means to fulfil tree nutrient requirements as HLB obstructs nutrient flow via phloem reducing symptoms by about 40% (Morgan et al., 2016; Pustika et. al., 2008). These findings support the belief that HLB affected trees are limited in soil nutrient uptake; otherwise, foliar applied nutrients might extend tree life and increased yield as close as to those obtained prior to the incidence of HLB (Pustika et al., 2008; Rouse et al., 2010). The impact of rate and method of application of foliar nutrition on tree water and nutrient uptake and accumulation on HLB affected citrus trees has been lacking. Therefore, the objective of this study was to determine whether nutrient accumulation and water uptake of HLB affected *Citrus sinensis* 'Valencia' trees were affected by rootstocks at selected soil and foliar rates of essential nutrients.

Materials and Methods

Site conditions. The study was carried out at the University of Florida, Southwest Florida Research and Education Center (SWFREC) Immokalee, FL (26.42° N and 81.43° W, at 34 ft

The authors are grateful to Florida Citrus Initiative for generously funding this project.

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above sea level). The first study was performed using 'Valencia' trees budded on *C. volkameriana* Pasquale (Volk) and Swingle citrumelo {(*Citrus × paradisi* Macf.) × [*Poncirus trjfoliata* (L.) Raf.]} rootstocks at 12 ft and 11 ft distance between trees in the planted row, respectively, 25, and 21 ft between tree rows on 45- and 45-ft beds width, respectively. The trees were planted in Apr. 2006, on Immokalee fine sand series, consists of nearly level, poorly drained soils on the flatwoods formed in sandy marine sediments with slopes < 2% (Obreza and Collins, 2008) classified as sandy, siliceous, hyperthemic Arenic Haplaquods with the Spodic horizon lying within 1m from the ground surface (USDA, 1990).

Treatments and experimental design. The experiment was set up in a split-split plot design consisting of two types of rootstocks, three N rates (150, 200, and 250 lb/acre), and single foliar rate Zn, Mn, and B, and /or soil applied sulfur encapsulated Mn and Zn tiger product (Table 1). Each plot contained 12–20 trees from which four trees were selected for data collection with six plots per block.

Experimental plots received one of the following treatments. Treatment 1 (T1) was the untreated control; trees received neither soil nor foliar micronutrient application. Treatment 2 (T2) received only foliar applied micronutrients. Treatment 3 (T3) received both foliar and soil micronutrients at the same rate and timing as T2. Treatment 4 (T4) received foliar applications at the same rate and timing as T2 and soil applications twice as high as the foliar rate (Table 1). Treatments resulted in applications of 0× (untreated control), 1× (foliar only), 2× (1× foliar and 1× soil applied), and 3× (1× foliar and 2× soil applied) of the amounts of previous recommended (Obreza and Morgan, 2008).

Table 1. Soil and/or foliar applied nutrients to *Citrus sinensis* 'Valencia' trees at the Concept Grove at University of Florida SWFREC/IFAS, Immokalee, FL during the 2016, 2017, and 2018 growing seasons.

			Method of application					
	Soil		Foliar (lb/acre/yr)			Soil (lb/acre/yr)		
Treatment	N rate	K rate	Zn[z]	Mn[y]	B[x]	Zn[w]	Mn[w]	B[v]
1	150[u]	150[t]	–	–	–	–	–	–
	200[s]	150	–	–	–	–	–	–
	250[r]	150	–	–	–	–	–	–
2	150	150	8	8	2	–	–	–
	200	150	8	8	2	–	–	–
	250	150	8	8	2	–	–	–
3	150	150	8	8	2	8*	8*	2*
	200	150	8	8	2	8	8	2
	250	150	8	8	2	8	8	2
4	150	150	8	8	2	16**	16**	4**
	200	150	8	8	2	16	16	4
	250	150	8	8	2	16	16	4

[z]Foliar spray 0.09 lb/gal $ZnSO_4$ per acre equivalent.

[y]Foliar spray 0.11 lb/gal $MnSO_4$ per acre equivalent.

[x]Foliar spray 0.01 lb/gal B $Na_2B_4O_7$ per acre equivalent.

[w]Soil applied sulfur encapsulated 6% $ZnSO_4$ and 6% $MnSO_4$ 0.35 lb and 0.70 lb. per plot (each plot = 0.09 acre) for Treatment 3(*) and Treatment 4(**), respectively

[v]Soil applied equivalent to 0.33 and 0.66 lb of $Na_2B_4O_7$ per plot (each plot = 0.09 acre) for Treatment 3(*) and Treatment 4(**), respectively.

[u]150 lb. N per acre as KNO_3 split 20 times per year.

[t]150 lb. K per acre as NH_4NO_3 split 20 times per year.

[s]200 lb. N per acre as KNO_3 split 20 times per year.

[r]250 lb. N per acre as KNO_3 split 20 times per year.

Treatments were randomly assigned across the plots. The three N rates were applied as split application two times per month from February to November resulting in 20 applications per year. Treatments were applied three times annually matching early spring, summer, and late summer flushes (Hall and Albrigo, 2007) using truck mounted sprayer (Hypro corporation, New Brington, MN). Plants were irrigated daily with micro-sprinkler placed at about 6 inches perpendicular to the tree row and irrigation duration was determined by smart irrigation apps for androids <http://smartirrigationapps.org/>. The micro-sprinkler irrigation was supplied with single 10 gal/h Max-14 (Maxijet, Dundee, FL) fill-in blue emitter for micro-sprinkler at each tree (Kadyampakeni et al., 2016; Schumann et al., 2009).

Tissue sampling and analysis. Four- to six-month-old leaves were randomly collected across four quadrants (north, south, east and west) of each tree. The leaves were collected from non-fruiting branches located at approximately 2/3 the height of the canopy in spring and summer season each year (Obreza et al., 2010). The leaf samples were washed in weak detergent solution, rubbed between the thumb and forefinger, subsequently rinsed with reverse osmosis water followed by deionized water to remove nutrients adhering to the leaf surface (Engles et al., 2000; Obreza et al., 2008). The leaf samples were oven dried at 65 °C until constant dry matter attained and subsequently ground to pass through 20–60 mesh screen as described in Engles et al., 2000 and Jones and Case, 1990. A sample of 0.5 g dry tissue was weighed and subjected to 500 °C for 16 h to dry ash. The ashed samples were dissolved with 15 mL of 0.5 M HCl at room temperature for 1/2 h. The solutions were transferred into 15-mL glass tubes and placed in a refrigerator at ≤ 4 °C pending analyses by inductively coupled plasma-optical emission spectrometer (ICP-OES) (Spectro Ciros CCD, Fitchburg, Mass.) (Kadyampakeni et al., 2016; Morgan et al., 2006). The NA2500 carbon analyzer (Thermoquest CE Instruments; Thermoquest Corporation, Thermo Fisher Scientific Inc., Waltham, Mass.) was utilized to determine tissue nitrogen (N). Subsequently, nutrients were compared to the ranges of CCFCN (Obreza et al., 2008).

Stem water potential. Stem water potential (stem ψ_ω) measurements were conducted on three mature leaves per plant and four plants per treatment in spring and summer of each year. Each leaf was covered with transparent plastic bag followed by aluminum foil (Barkataky et al., 2013; Naor, 2000). After an equilibration period of 24 h, each leaf, including the petiole, was cut from the shoot, and the stem water potential was estimated directly in the field with a pressure chamber (Model 3005 plant water status console; Soil Moisture Equipment Corporation, Santa Barbara, Calif.) (Kadyampakeni and Morgan, 2017; Naor, 2000). Leaf area index (LAI) was determined by means of a portable LAI meter (Model LI-3000A LI-COR, Lincoln, NE). The LAI of each plot was estimated using a SunScan canopy device (Dynamax Inc., Houston, TX) during a sunny day as per recommendation of the manufacturer (Kadyampakeni et al., 2014b; Hamido et al., 2017). The LAI was determined by taking the average of the east–west and north–south readings.

Statistics and data analysis. Data of leaf analysis and stem ψ_ω were analyzed using repeated measures analysis (PROC GLM Mixed Model procedures, SAS 9.4,0 SAS Institute, Cary, N.C.). Data subjected to spatial and temporal variability were tested for qualitative statistical model assumptions of linearity, normality, homogeneity of variance, and independent errors. The Tukey-Kramer Grouping Range Test was used to compare the means for treatments with F-tests with $P \leq 0.05$. We also tested if there were interactions between LAI and stem ψ_{ω} to determine model description and coefficient of determination on the parameters.

Result and Discussion

Leaf nutrient concentration. During both seasons of the first year (2016), leaf N was below the range of the critical concentration for Florida nutrient concentration (CCFCN) regardless of the N rate (Fig. 1A and Fig. 1B). The delay in response to the split N application was attributed to the earlier observation that N absorbed by trees first accumulates in the woody tissue of the tree (Morgan and Hanlon, 2006). The large canopy volume of the trees in this study needed prolonged time to respond to the N application. In Spring 2017, even though there was no significant difference among the treatments, leaf N concentration was higher, within the range of CCFCN with lower N rate potential requirement than those recommended for healthy trees (Obreza and Morgan, 2008) (Fig. 1C and Table 3). In Summer 2017, leaf N was above the range of the CCFCN with significantly lower concentration on Volk rootstock than Swingle rootstocks treated at the lowest N rate; the variation between rootstocks was

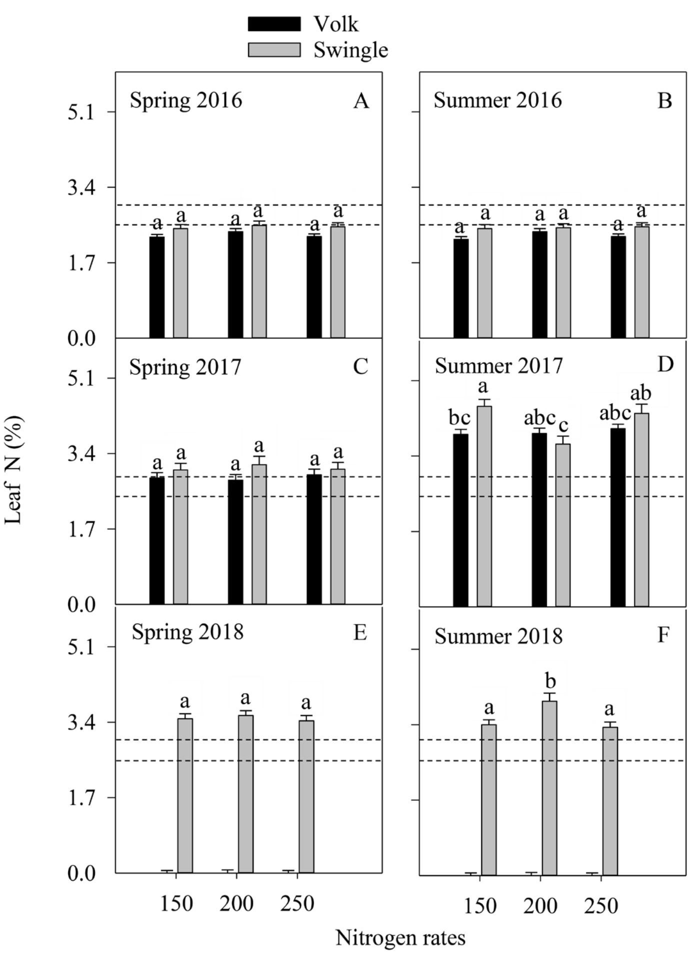

Figure 1. Leaf N concentration of *Citrus sinensis* 'Valencia' trees. Bars with different lowercase letters represent significantly different at $P < 0.05$ using the Tukey-Kramer HSD test. Dashed lines indicate the optimum range of critical concentration for Florida citrus nutrition. As Hurricane Irma removed trees budded on Volkameriana rootstocks in Sept. 2017, data presented only for trees budded on Swingle rootstock during Spring and Summer 2018 (Fig. 1E and 1F).

eliminated with the increasing N rates (Fig. 1D). However, the apparent lower leaf N concentration with higher N treatment rates as compared with the lower N treatment rate was because of the dilution effect caused by the massive tree biomass accumulation following the spring flush (data not shown). As Hurricane Irma eliminated trees on Volk rootstock, leaf N concentration was above the range of the CCFCN during 2018 of both seasons on trees budded on Swingle rootstock (Fig. 1E and F). During Summer 2018, leaf N concentration was significantly higher when trees received the moderate N rate (200 lb. acre^{-1}). Yet, the study indicated that lower N rate potential requirement for HLB affected citrus trees with N split applications.

Leaf Mn concentration was below the CCFCN in trees under the control treatment during the three years period of study, with significantly lower leaf Mn concentration in the control than citrus trees receiving Mn applications, except at the beginning of the experiment in the spring of 2016 (Table 2 and Table 3). This result was similar to leaf analysis reported on young and matured HLB affected grapefruit, *Citrus* × *paradisi* (Tian et al., 2014), *C. sinensis* 'Valencia' (Auber, 1988; Morgan et al., 2016); *C. sinensis* 'Hamlin (Auber, 1988; Spann and Schumann, 2009b), and other citrus species (Masaoka et al, 2011). Leaf Mn concentration of trees receiving T2, T3 and T4 satisfied the range of the CCFCN, while T3 and T4 were not significantly different during spring seasons as compared with T2. This implied that soil-applied micronutrients were not necessary under well-managed N and K split application, and foliar Mn sprays. However, trees receiving T4 showed significantly higher leaf Mn concentration than T2 and T3 in Summer 2017 (B). At the end of the third year (Summer 2018), trees that received T4 had significantly the lower leaf area index values (data not shown), indicating reduction in growth, hence inferring excessive nutrient application.

Except in Spring 2016, leaf Zn concentration values, like leaf Mn concentration values, were below the range of CCFCN in the control treatment during the three years of this study (Table 2). Even though leaf Zn concentration of T2, T3 and T4 satisfied the range of the CCFCN, T3 and T4 were not significantly different as compared with T2 during spring and summer seasons of the entire experiment. This inferred that soil-applied Zn did not influence leaf Zn concentration and was less mobile in the tree as compared to leaf Mn of its counterpart T4. However, when the trees were severely defoliated because of Hurricane Irma and extended flooding one or two time soil applied Mn and Zn could be recommended (Table 2E and F). Leaf B concentration remained above the range of the CCFCN regardless of the treatment application and season of the year during this study. Similar results have also been reported on the first two years of five year studies on leaf B concentration on HLB affected 'Valencia' (Morgan et al., 2016; Spann and Schumann, 2009b), and 'Hamlin' citrus trees (Spann and Schumann, 2009b).

Leaf P and K were within the range of the CCFCN, but leaf K concentrations were influenced by dilution because of increased biomass (data not shown) (Table 2B and Table 2D). Leaf Ca, Mg, and Fe also remained within the range of the CCFCN but leaf Ca concentration increased over time. However, leaf Mg concentration was deterred as Zn and Mn concentrations increased late in the experiment (Table 2E and Table 2F). Leaf Cu concentrations were significantly higher than the range of the CCFCN because of uptake of Cu from foliar applied pesticide products and also because of decreasing soil pH level caused by sulfur encapsulated metallic Zn and Cu products.

Table 2. Leaf nutrient concentration on dry weight basis of *Citrus sinensis* 'Valencia' trees at the University of Florida grove near Immokalee, FL. the during 2016, 2017, and 2018 seasons.

Rootstock	Micro[1]	P	K	Ca	Mg	Zn	Mn	B	Cu	Fe
A					Spring 2016					
		<----(%)----			---->	<----(ppm)----				---->
Volk	1	0.16 ab	2.13	3.04	0.21	108.8	229.4	187.1 a[2]	295.3	63.5
Volk	2	0.13 b	2.31	2.96	0.21	132.1	248.7	134.3 b	311.3	63.6
Volk	3	0.13 b	2.33	2.84	0.20	128.5	256.3	171.2 ab	289.2	65.1
Volk	4	0.14 ab	2.47	2.75	0.19	133.0	252.3	170.9 ab	306.6	58.0
Swingle	1	0.16 ab	2.17	2.72	0.16	134.3	279.5	154.5 ab	322.9	44.4
Swingle	2	0.17 a	2.32	2.61	0.17	146.2	269.5	139.3 ab	321.8	49.8
Swingle	3	0.15 ab	2.33	2.50	0.16	133.7	261.5	173.6 ab	294.4	43.0
Swingle	4	0.16 ab	2.31	2.62	0.17	141.9	281.8	159.1 ab	326.3	44.0
B					Summer 2016					
Volk	1	0.15 ab	1.77 abc	3.52	0.25	30.6 b	89.4 c	159.3	111.7	61.5 a
Volk	2	0.14 ab	2.02 a	3.18	0.24	103.6 a	136.4 ab	167.0	122.4	65.0 a
Volk	3	0.14 b	1.95 ab	3.22	0.23	159.7 a	139.0 ab	177.2	124.6	66.2 a
Volk	4	0.14 b	2.04 a	3.25	0.23	150.0 a	126.6 b	187.6	118.3	62.6 a
Swingle	1	0.16 ab	1.39 c	3.28	0.22	36.3 b	101.3 bc	166.5	127.6	49.5 b
Swingle	2	0.17 a	1.50 bc	3.34	0.21	108.3 a	166.0 a	184.6	153.2	53.7 b
Swingle	3	0.16 ab	1.62 abc	3.28	0.21	157.1 a	179.6 ab	177.4	150.4	51.5 b
Swingle	4	0.16 ab	1.58 abc	3.30	0.22	159.2 a	168.3 a	174.2	148.5	51.9 b
C					Spring 2017					
Volk	1	0.21	1.67	5.10	0.25	23.4 d	29.4 d	178.0 b	31.4 abc	64.6 ab
Volk	2	0.21	1.93	5.01	0.26	101.5 bc	93.3 bc	202.4 ab	30.1 c	66.7 b
Volk	3	0.20	2.66	4.90	0.25	128.5 c	127.1 c	206.5 ab	31.3 ab	65.5 b
Volk	4	0.22	3.86	4.86	0.25	131.5 ab	194.3 ab	213.0 a	32.1 a	63.0 ab
Swingle	1	0.20	2.29	5.06	0.26	17.4 d	26.5 d	201.1 ab	26.0 c	69.8 a
Swingle	2	0.20	2.42	4.91	0.23	107.1 c	110.3 c	206.2 ab	25.5 c	66.2 a
Swingle	3	0.20	2.83	5.01	0.23	107.9 ab	115.6 bc	186.1 ab	23.8 bc	63.6 b
Swingle	4	0.19	1.79	4.92	0.23	167.0 a	180.9 a	198.8 ab	23.6 bc	64.3 b
D					Summer 2017					
Volk	1	0.14	1.49	3.46 b	0.19 bcd	23.4 b	16.8 a	149.1	97.7	74.7 a
Volk	2	0.14	1.58	3.36 b	0.19 cd	74.6 a	47.9 bc	154.7	113.2	54.7 ab
Volk	3	0.14	1.45	3.34 b	0.18 d	92.7 a	97.6 b	161.5	106.5	58.6 ab
Volk	4	0.14	1.43	3.43 b	0.19 cd	92.1 a	223.2 a	160.5	106.7	61.0 ab
Swingle	1	0.17	1.30	3.75 ab	0.24 a	26.7 b	14.6 a	192.7	85.7	43.4 b
Swingle	2	0.17	1.16	3.98 a	0.24 a	102.8 a	69.5 bc	182.7	92.7	55.8 ab
Swingle	3	0.15	1.28	4.05 a	0.22 ab	106.4 a	119.7 b	181.1	90.7	54.1 ab
Swingle	4	0.16	1.60	3.71 ab	0.21 abc	109.4 a	192.2 a	182.2	78.0	44.6 ab
E					Spring 2018					
Swingle	1	0.21	1.16	4.73	0.33	4.3 c	4.5 b	115.5	46.1	31.6
Swingle	2	0.21	1.31	4.50	0.31	3.9 bc	29.7 b	111.3	38.7	27.0
Swingle	3	0.19	1.14	4.87	0.29	12.4 ab	176.8 a	140.5	49.4	31.0
Swingle	4	0.19	1.13	4.46	0.28	14.2 a	244.2 a	127.9	46.3	28.6
F					Summer 2018					
Swingle	1	0.16	1.22	4.15	0.27 a	31.89 b	19.8 b	103.7	54.2	56.0
Swingle	2	0.17	1.28	4.05	0.27 ab	64.10 a	54.1 b	118.3	60.8	65.8
Swingle	3	0.15	1.27	4.12	0.27 b	63.57 a	155.0 a	116.9	54.1	64.8
Swingle	4	0.17	1.27	3.93	0.26 b	70.33 a	115.8 a	106.7	58.5	78.0

[1]Nutrient treatments: 1 = control, 2 = foliar (1×), 3 = foliar (1×) and soil (1×), and 4 = foliar (1×) and soil (2×), (1× = 8 lb/acre of each of Zn and Mn and 2 lb/acre of B).

[2]Mean within the same column followed by different letters were significant at $P < 0.05$ using the Tukey-Kramer HSD test. As Hurricane Irma removed trees budded on Volkameriana in Sept. 2017, data were presented only for trees budded on Swingle rootstock during Spring and Summer 2018 (Tables 2E and 2F).

Table 3. ANOVA of soil and/foliar applied micronutrients, nitrogen rate, and rootstock effect on leaf nutrient concentration and stem water potential (ψ_ω) of *Citrus sinensis* 'Valencia' trees at the University of Florida grove near Immokalee, FL during the 2016–2018 growing seasons.

Effect[z]	N	P	K	Ca	Mg	Zn	Mn	B	Cu	Fe	ψ_ω	N	P	K	Ca	Mg	Zn	Mn	B	Cu	Fe	ψ_ω
A					Spring 2016											Summer 2016						
	<-- Significance[y] -->																					
R	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	***	NS	*	*	NS	NS	NS	NS	NS	NS	***	***
M	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	*	NS	NS	*	*	NS	NS	NS	NS
N	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**
R×M	NS	NS	NS	NS	NS	NS	NS	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
R×N	NS	NS	NS	NS	NS	NS	NS	***	NS	NS	**	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
N×M	NS	NS	NS	NS	NS	NS	NS	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
R×M×N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
B					Spring 2017											Summer 2017						
	<-- Significance -->																					
R	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	***	NS	NS	NS	***	***	NS	NS	NS	NS	NS	***
M	NS	NS	NS	NS	NS	***	***	NS	***	***	**	NS	NS	NS	NS	**	***	***	NS	NS	NS	NS
N	ns	NS	NS	NS	NS	NS	NS	NS	***	***	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
R×M	NS	NS	NS	NS	NS	NS	NS	NS	**	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
R×N	NS	NS	NS	NS	NS	NS	NS	NS	***	*	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
N×M	NS	NS	NS	NS	NS	NS	NS	*	***	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
R×M×N	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
C					Spring 2018											Summer 2018						
	<-- Significance -->																					
M	NS	NS	NS	NS	**	***	***	NS	NS	NS	NS	**	NS	NS	NS	NS	***	***	NS	NS	NS	NS
N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
M×N	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Factorial effects: R = rootstocks, M = micronutrients, and N = nitrogen rate.

[y]Significance: NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and < 0.0001, respectively. As Hurricane Irma removed trees budded on Volkameriana rootstocks in Sept. 2017, ANOVA presented only for trees budded on Swingle rootstock during Spring and Summer 2018 .

Stem water potential. Significantly lower stem ψ_ω was observed on trees budded on Volk rootstocks than Swingle rootstocks regardless of nutrient treatment during Spring 2016 (Fig. 2A and Table 3). Similar results had been recorded in the summer of the same year with significantly lower stem ψ_ω on trees budded on Volk rootstock than Swingle rootstocks on all the treatment except on T3 (Fig. 2B). On average, 0.70 ± 0.03 and 0.8 ± 0.03 stem ψ_ω were recorded on trees budded on Volk and Swingle rootstocks, respectively. Hamido et al., (2017) reported similar results on HLB affected citrus during dry season and increased stem ψ_ω during wet season at three different sites. During Spring 2017, significantly lower stem ψ_ω was observed on trees budded on Swingle than Volk rootstocks except on trees that received T2 (Fig. 2C). Similar results were noted during Summer 2017 with significantly lower stem ψ_ω on trees budded on Swingle rootstocks than Volk rootstocks (Fig. 2D). Increasing stem ψ_ω could be because of the increase in leaf area index (LAI, data not shown) pertinent to the treatment effect. Thus, we tried to determine if LAI could be related to the stem ψ_ω. The results indicate negative relationship between the LAI and stem ψ_ω ($R^2 = 0.55$, n = 360) in 366 observations (Fig. 3). This notion might be elucidated by the phenomenon of soil–plant-atmosphere continuum, in which trees with high LAI were exposed to greater transpiration driving forces such as light intensity and wind speed thereby affecting stem ψ_ω. In a study conducted on *Citrus reticulata* Blanco, stem ψ_ω and leaf ψ_ω showed significantly higher correlation with stomatal conductance resulting in $R^2 = 0.74$ and 0.62, respectively (Sdoodee and Somjum, 2008).

Conclusion

This study indicated that constant split N application for one year brought mature HLB affected N deficient citrus to the optimum ranges of CCFCN, with lower N rate potential requirement. Nitrogen rates lower than the rate used in the current study can be a potential study area in a well-established citrus groves or in younger citrus trees. This study indicated that foliar application of Mn and Zn nutrients were enough to satisfy citrus crop requirement. However, after massive vegetative loss caused by a hurricane and leaching rain events, a one-time soil application of Zn and Mn might be necessary. No effect of applied B on leaf B concentration could be determined as leaf B concentration was higher than CCFCN. Water stress level was not a major problem hence we could not observe water uptake variation due to treatment indicating that daily irrigation scheduled using SmartIrrigation assisted to maintain the water lever within the field capacity.

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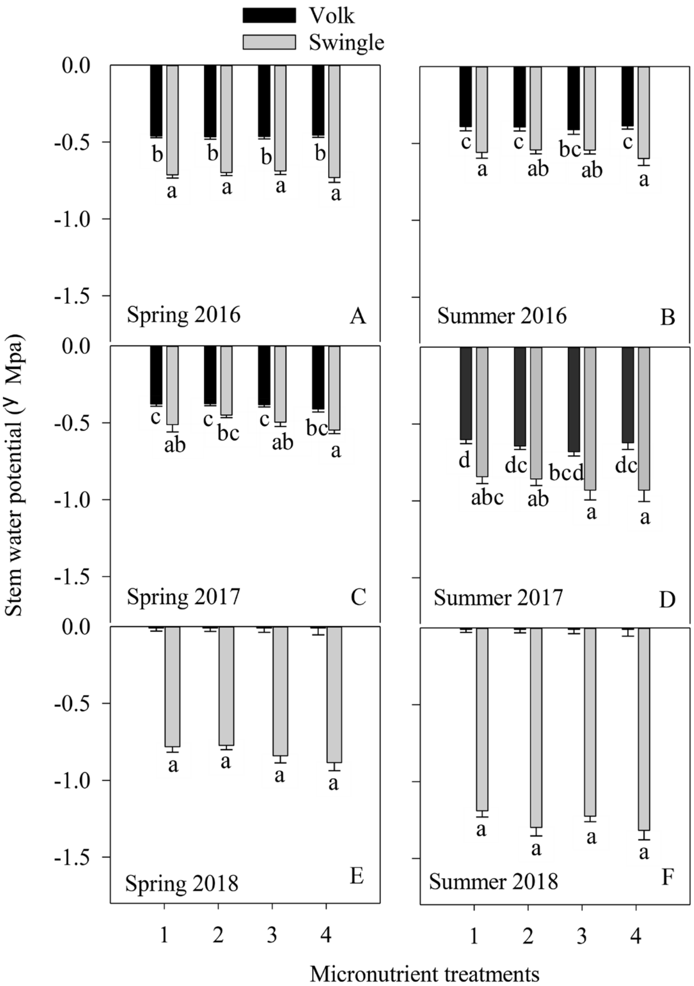

Fig. 2. Stem water potential (yω) of *Citrus sinensis* 'Valencia' trees. Treatments: (1) control, (2) foliar (1×), (3) foliar (1×) and soil (1×), and (4) foliar (1×) and soil (2×), (1× = 8 lb. acre^{-1} of Zn and Mn each and 2 lb. acre^{-1} of B). Bars with different lowercase letters represent significantly different at $P < 0.05$ using the Tukey-Kramer HSD test. As Hurricane Irma removed trees budded on Volkameriana rootstocks in Sep. 2017, data presented only for trees budded Swingle rootstock during spring and summer 2018 (Fig. 2E and 2F).

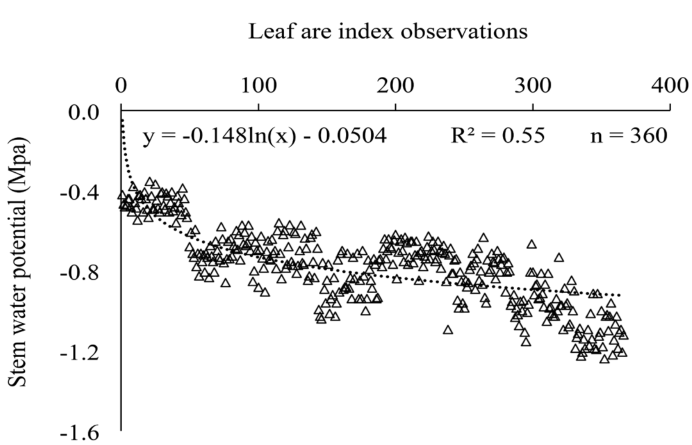

Fig. 3. Logarithmic correlation between stem water potential and leaf area index on *Citrus sinensis* 'Valencia' trees.

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