

Foliar Applications of Essential Nutrients on Growth and Yield of ‘Valencia’ Sweet Orange Infected with Huanglongbing

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Abstract. Huanglongbing (HLB) causes citrus root systems to decline, which in turn contributes to deficiencies of essential nutrients followed by decline of the canopy and yield. This study was conducted on a 6-year-old ‘Valencia’ [*Citrus sinensis* (L.) Osb.] on Swingle rootstock (*Citrus paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.) trees in a commercial grove near Immokalee, FL, to evaluate the effects of foliar applications of selected essential nutrients (N, K, Mn, Zn, B, and Mg) on growth and productivity of citrus trees infected with *Candidatus Liberibacter asiaticus* (CLAs), the pathogen putatively associated with HLB in Florida. Mn, Zn, B, and Mg were applied in all experiments to drip at 0×, 0.5×, 1.0×, and 2.0×/spray of what has been traditionally recommended in Florida to correct deficiencies. Treatments were applied foliarly 3×/year with the sprays occurring during each growth flush for 5 years (2010–14). Thus, the 0×, 0.5×, 1.0×, and 2.0×/spray treatments resulted in 0×, 1.5×, 3.0×, and 6.0×/year to correct deficiencies. MnSO₄ and ZnSO₄ were applied with or without KNO₃ and in separate experiments were compared with Mn₃(PO₃)₂ and Zn₃(PO₃)₂, respectively. Disease incidence, foliar nutrient content, canopy volume, and yield were measured. At the beginning of the experiment, foliar N, P, Ca, Mg, Cu, and B were in the sufficient range and K, Mn, Zn, and Fe were slightly low. Disease incidence was very high with 83% and 98% of trees testing positive for CLAs in 2010 and 2014, respectively. Nutrients that are not mobile or have limited mobility in plants, namely Mn, Zn, and B, demonstrated an increase in foliar concentration immediately after spray and in the annual averages. Foliar K increased from the deficient to the sufficient level by KNO₃ sprays, but the mobile nutrients N and Mg did not show an increase in foliar levels, indicating that intraplant transport occurs in the presence of HLB. Foliar KNO₃ application had a stronger effect on growth than yield. Yield was most strongly affected by application of MnSO₄ where yield of the 3×/year treatment was 45% higher than that of the unsprayed control, but yield declined by 25% for the 6×/year treatment. Yield within 95% of the maximum occurred with foliar Mn concentrations of 70–100 μg·g⁻¹ dry weight when Mn was applied as MnSO₄, which is at the high end of the traditionally recommended 25–100 μg·g⁻¹ dry weight range. The phosphite form of Mn [Mn₃(PO₃)₂] depressed yield by an average of 25% across all application concentrations. Zn, B, and Mg did not significantly impact yield. Canopy volume demonstrated concave relationships across application concentrations for MnSO₄ and ZnSO₄ without KNO₃ and Mn₃(PO₃)₂, Zn₃(PO₃)₂, Boron, and MgSO₄ with KNO₃, with the minimum occurring near the 3×/year application concentration. These data indicate a complex interaction in the amount of nutrients applied and their corresponding effects on foliar concentration, growth, and yield for HLB-affected trees. The results of this study at least partially explain the current confusion among scientists and the commercial industry in how to manage nutrition of HLB-affected citrus trees. The traditionally recommended approaches to correcting nutrient deficiencies need to be reconsidered for citrus with HLB.

HLB (citrus greening) is the most devastating citrus diseases in many parts of the world and is putatively caused by CLAs, a nonculturable, phloem-limited bacteria (Bové 2006; Gottwald, 2010; Subandiyah et al., 2000). The disease is widespread in Florida, Texas, Brazil, Mexico, and other major producing areas throughout the world causing significant concerns about the economic viability of these citrus industries (Bové 2006; Gottwald, 2010; Spann and Schumann 2009; Subandiyah et al., 2000).

HLB was identified in Florida in 2005, and by 2007, it had significantly reduced yields in citrus groves in the state, devastating the \$9 billion per year industry (Gottwald et al., 2007; Irely et al., 2006, 2008; Manjunath et al., 2008). By 2015, about 80% of citrus trees in Florida were infected with the HLB pathogen (Singerman and Useche, 2016) making tree removal to reduce the source of inoculum impractical (Gottwald, 2010) and a need for alternative management practices imperative until resistant trees are developed

or the principle vector, the Asian citrus psyllid (ACP), is eradicated.

Visual HLB symptoms of citrus usually start as chlorosis of a single branch, which then spreads to the rest of the tree (Garnier and Bové, 2000). Different types of chlorosis develop, including interveinal chlorosis of young leaves, similar in symptomology to Zn and Mn deficiencies that develop early in the growing season, followed by amorphous mottling of older leaves, which develop later in the growing season and have been associated with over production of starch that disrupts the grana in chloroplasts (Achor et al., 2010; Etxeberria et al., 2009; Kim et al., 2009). Shoot tips can also develop a rosette pattern (shortened internodes) and small leaves similar to Zn deficiency (Bové, 2006; da Graça, 1991). Nutrient deficiencies have been shown to develop in HLB-affected trees, including Mn, Zn, P, Ca, Mg, and Fe (Aubert, 1979; Handique et al., 2012; Rouse et al., 2012; Spann and Schumann, 2009). HLB causes roots, in particular fibrous roots, to decline within a few months after infection (Graham et al., 2013; Johnson et al., 2013; Kadyampakeni, 2012; Kadyampakeni et al., 2014a, 2014b) and before foliar symptoms develop (Graham et al., 2013). Fibrous roots are responsible for the bulk of nutrient uptake and their decline likely explains the deficiency symptoms that develop in the canopy (Pustika et al., 2008). In severely affected trees, fruit become bitter from low acidity and production of bitter compounds, peel disorders develop, growth is stunted, and fruit can become misshapen and drop prematurely reducing yields (Baldwin et al., 2014; Bassanezi et al., 2009; Dagulo et al., 2010; Gottwald et al., 2007).

Research that demonstrated that HLB symptoms could be reduced by foliar applications of micronutrients, especially Mn, Zn, B, and Mg, and other physiologically active compounds, such as salicylic acid and phosphite (Pustika et al., 2008; Shen et al., 2013; Stansly et al., 2014), have promoted development and use of enhanced foliar nutritional programs in the commercial industry in Florida. Efficacy of these programs has been a topic of considerable discussion and debate with no clear consensus on the best approach to alleviating symptoms in commercial groves. Fertilization programs have varied considerably among growers, and have consisted of various rates and application schedules of essential macro- and micronutrients with some programs including salicylate and phosphite salts. Although some studies have shown benefits of enhanced nutritional programs (Pustika et al., 2008; Shen et al., 2013; Stansly et al., 2014), at least one has not (Gottwald et al., 2012). HLB causes declines in the canopy and root system (Graham et al., 2013; Johnson et al., 2013; Kadyampakeni, 2012; Kadyampakeni et al., 2014a, 2014b) and since leaves typically persist up to 2 years in citrus (Wallace et al., 1954), it would be expected that alleviation of disease symptoms would require a 2-year window to rebuild the root system and canopy before

Table 1. Treatments applied to 'Valencia' trees at the University of Florida, Southwest Florida Research and Education Center, Immokalee, FL, from 2010 to 2014.

Expt. ²	Chemicals applied	Application rate		Nutrient concn		
		Rate/spray compared with recommended ³	Rate/yr compared with recommended ³	(mm·L ⁻¹ spray)	(kg·ha ⁻¹ ·yr ⁻¹)	
1	No spray	—	—	—	—	
	KNO ₃ ^x	—	—	685	56	
2	KNO ₃	MnSO ₄	0.5×	1.5×	33	2
	KNO ₃	MnSO ₄	1.0×	3.0×	65	4
	KNO ₃	MnSO ₄	2.0×	6.0×	131	8
	—	MnSO ₄	0.5×	1.5×	33	2
	—	MnSO ₄	1.0×	3.0×	65	4
3	—	MnSO ₄	2.0×	6.0×	131	8
	KNO ₃	ZnSO ₄	0.5×	1.5×	37	3
	KNO ₃	ZnSO ₄	1.0×	3.0×	73	6
	KNO ₃	ZnSO ₄	2.0×	6.0×	147	11
	—	ZnSO ₄	0.5×	1.5×	37	3
4 ^w	—	ZnSO ₄	1.0×	3.0×	73	6
	—	ZnSO ₄	2.0×	6.0×	147	11
	KNO ₃	Mn ₃ (PO ₃) ₂	0.5×	1.5×	33	2
	KNO ₃	Mn ₃ (PO ₃) ₂	1.0×	3.0×	65	4
	KNO ₃	Mn ₃ (PO ₃) ₂	2.0×	6.0×	131	8
5 ^v	KNO ₃	Zn ₃ (PO ₃) ₂	0.5×	1.5×	37	3
	KNO ₃	Zn ₃ (PO ₃) ₂	1.0×	3.0×	73	6
	KNO ₃	Zn ₃ (PO ₃) ₂	2.0×	6.0×	147	11
	KNO ₃	B	0.5×	1.5×	11	1.4
6	KNO ₃	B	1.0×	3.0×	22	3
	KNO ₃	B	2.0×	6.0×	44	6
	KNO ₃	MgSO ₄	0.5×	1.5×	296	8
7	KNO ₃	MgSO ₄	1.0×	3.0×	592	17
	KNO ₃	MgSO ₄	2.0×	6.0×	1,184	34

²Treatments 2, 3, 6, and 7 included the respective no spray control and/or KNO₃ only treatment as a control where appropriate.

³Taken from Obreza and Morgan (2008).

^xAll KNO₃ treatments received the same application rate. There is currently no recommended rate for foliar applications of KNO₃.

^wIncluded the KNO₃ + MnSO₄ treatments.

^vIncluded the KNO₃ + ZnSO₄ treatments.

yield would recover. It appears that longer term studies with foliar applications of Mn, Zn, B, and Mg are required with the twin goals of first aiding recovery of the root systems and the canopy followed by recovery of yield.

Some growers in the Florida citrus industry are using phosphite as the anionic complement to the cationic micronutrients in the salts used to apply essential nutrients to the foliage, such as Mn₃(PO₃)₂ and Zn₃(PO₃)₂. Early studies of phosphites use in agriculture involved evaluating its nutritional (Rickard, 2000) and other horticultural benefits, including an increase in citrus flowering, fruit set, and yield, and higher fruit quality (Rickard, 2000). More recently, however, the beneficial uses of phosphites on plants have focused on its control of pathogenic

fungi of plants (Deliopoulos et al., 2010) including citrus fungal diseases (Afek and Szejnberg, 1989; Agostini et al., 2003; Dick and Ramsfield, 2011; Gutter, 1983; Orborvic et al., 2008; Yogev et al., 2006; Zhu et al., 1993). Phosphites apparently inhibit fungi by disrupting biosynthesis of polysaccharides, lipids, nucleic acids, and proteins (Niere et al., 1994), by inhibiting adenylate synthesis (Griffith et al., 1990), and by inhibiting phosphorylating enzymes involved in metabolic pathways such as glycolysis and pentose phosphate metabolism (Barchietto et al., 1992; Stehmann and Grant, 2000). The lack of similar metabolic inhibitions by phosphite in plants may be related to its transport and accumulation in the vacuole when phosphite is not deficient (Danova-Alt et al., 2008). However, when phosphate is deficient, phosphite accumulates in the cytoplasm (Danova-Alt et al., 2008) where it has a deleterious effect on plant metabolism (McDonald et al., 2001). Phosphite is readily absorbed by plant leaves and translocated throughout the plant via the phloem and xylem (Guest and Grant, 1991). Phosphite can be converted to phosphate by soil bacteria (McDonald et al., 2001), but whether CLAs can convert it is unknown. Little is known about the bactericidal properties of phosphites. The research that demonstrated beneficial uses of phosphites on citrus occurred before the HLB pandemic; how HLB-infected citrus trees will respond to phosphites is largely unknown.

The goal of this study was to evaluate nutrient uptake, tree growth, and yield of HLB-affected citrus trees treated with various combinations of macro- and micronutrients, and with or without phosphite as the balance anion over a 5-year period. A specific goal of developing information needed to make recommendations for foliar nutrient applications was considered by applying macro- and micronutrients in selected combinations at three rates. The objectives of this study were to determine the effect of foliar nutrient applications on disease incidence, leaf nutrient concentrations, leaf nutrient status before and after nutrient foliar application, tree growth, and yields. This approach will provide the citrus industry with new information regarding fertilization practices to support continued production of existing citrus groves infected with HLB.

Materials and Methods

Site description. This study was initiated on 6-year-old 'Valencia' (*Citrus sinensis* Osb.) on Swingle rootstock (*Citrus paradisi* Macf. × *Poncirus trifoliata* Raf.) trees in a commercial grove near Immokalee, FL (lat. 26°25' N, long. 81°25' W). The trees were planted at a 3.9 × 6.8 m spacing on 14.7 m 1-wide beds (370 trees/ha) that were ≈1 m high and with drainage swales between every two rows. The trees had not filled their allotted space at the start of the experiment. The soil at the site was a nearly level, poorly

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drained Immokalee fine sand (sandy, siliceous, hyperthermic Arenic Haplaquods) with the spodic horizon lying within 1 m from the ground surface (Obreza and Collins, 2008). The trees were fertilized annually with soil applications of N–P–K at recommended rates (Obreza and Morgan, 2008).

Experimental designs and foliar applications of nutrients. Seven experiments were conducted

in this study (Table 1). Each experiment was conducted as a randomized complete block design with four blocks and two adjacent trees per block with data collected from both trees and averaged. Foliar applications of essential nutrients were applied by hand gun to drip. The application rate for KNO₃ was 685 mm·L⁻¹ and for each micronutrient were 0×, 0.5×, 1.0×, and 2.0×/spray of the current

recommended annual rates to correct deficiencies (Obreza and Morgan, 2008). Foliar applications were made 3×/year during the spring (March), early summer (June), and late summer (September) growth flushes. Thus, actual treatment applications were 0×, 1.5×, 3.0×, and 6.0×/year of the current recommendations. Applications were made every year from 2010 to 2014.

Table 2. Foliar nutrient concentrations, canopy volume, and yield of ‘Valencia’ trees that were not treated with any of the treatments as described in Table 1.

Yr	N	P	K	Ca	Mg	Mn	Zn	Cu	Fe	B	Canopy vol (m ³ /tree)	Yield (kg/tree)
	%			μg·g ⁻¹								
2010	2.79	0.17	1.1	3.3	0.34	17	23	66	46	163	—	—
2011	2.66	0.15	0.9	3.1	0.28	33	29	28	53	102	11.1	51
2012	2.45	0.13	1.3	3.1	0.26	19	24	13	46	87	14.3	50
2013	2.30	0.16	1.1	2.6	0.26	9	20	11	50	91	—	—
2014	2.58	0.11	0.9	2.3	0.19	31	55	13	40	73	15.5	106
Average	2.56	0.14	1.1	2.9	0.27	22	30	26	47	103	—	—
Optimum range	2.5–2.7	0.12–0.16	1.2–1.7	3.0–4.9	0.30–0.49	25–100	25–100	5–16	60–120	36–100	—	—

The foliar N means are for all trees that did not receive KNO₃, Mg are for all trees that did not receive foliar applications of MgSO₄, Mn are for trees that did not receive MnSO₄ or Mn₂(PO₃)₂, and so on.

Table 3. Response in foliar nutrition, growth, and yield of ‘Valencia’ trees with foliar applications of KNO₃.

	Foliar nutrient concn				Canopy vol (m ³ /tree)	Yield (kg/tree)
	Pre- vs. postapplication of KNO ₃ ^z		Annual avg ^y			
	N	K	N	K		
	(Pr > F)					
Overall model	0.20	<0.01	0.01	0.01	<0.01	0.02
Effects tested in model						
KNO ₃ × time ^x	0.37	0.19	—	—	—	—
Time	0.32	0.51	—	—	—	—
KNO ₃	0.50	0.80	0.95	0.02	0.06	0.35
Year	—	—	<0.01	<0.01	<0.01	0.04
Block	0.03	0.17	<0.01	0.02	<0.01	0.01
KNO ₃ × block	0.47	0.05	0.24	0.73	0.19	0.51
Main effect means						
Control	2.65	1.02	2.52	1.07b	13.6b	31.2
KNO ₃	2.70	1.24	2.52	1.27a	16.5a	37.3

The traditional optimum range for foliar N has been 2.5% to 2.7% and K is 1.2% to 1.7%.

^zPre- and postapplication measurements were made between 3 and 7 weeks apart. Pre- and postmeasurements were made twice in 2010 and once in 2012.

^yAverages were made of each treatment and rep for each year and used in the analysis.

^xTime = pre- vs. postapplication of KNO₃.

Table 4. Response in foliar levels of Mn, growth, and yield of ‘Valencia’ trees with foliar applications of MnSO₄.

	Foliar Mn concn		Canopy vol (m ³ /tree)	Yield (kg/tree)
	Pre- vs. postapplication of MnSO ₄ ^z			
	Annual avg ^y			
	(Pr > F)			
Overall model	<0.01	<0.01	<0.01	<0.01
Effects tested in model				
KNO ₃ × Mn rate × time ^x	0.64	—	—	—
KNO ₃ × time	0.18	—	—	—
Mn rate × time	0.01	—	—	—
Time	0.02	—	—	—
KNO ₃ × Mn rate ²	—	0.36	<0.01	0.39
KNO ₃ × Mn rate	—	0.28	<0.01	0.47
Rate ²	—	<0.01	0.87	<0.01
Rate	<0.01	<0.01	0.93	<0.01
KNO ₃	0.12	0.40	0.14	0.08
Year	—	0.01	<0.01	<0.01
KNO ₃ × block	0.66	0.93	0.03	0.95
Block	0.29	0.20	<0.01	<0.01
Main effect means				
Control	—	63.5	15.1	40.1b
KNO ₃	—	83.4	15.1	44.1a

The traditional optimum range for foliar Mn is 25 to 100 (μg·g⁻¹ dry weight).

^zPre- and postapplication measurements were made between 3 and 7 weeks apart. Pre- and postmeasurements were made twice in 2010 and once in 2012.

^yAverages were made of each treatment and rep for each year and used in the analysis.

^xTime = pre- vs. post-application of KNO₃.

Foliar nutrient analysis. Leaf samples were collected 1 to 2 weeks before treatment applications (preapplication) and 3–4 weeks after application (postapplication) to determine nutrient uptake using the procedures by Obreza and Morgan (2008) and processed according to tissue analytical methods (Hanlon et al., 1997; Jones and Case, 1990; Plank, 1992). A leaf sample of 50 recently expanded, mature leaves were randomly collected from the two trees in each treatment replication and placed in plastic bags and then in a cooler containing ice and taken to the laboratory at the University of Florida, Southwest Florida Research and Education Center (SWFREC) in Immokalee, FL. Leaf tissues were rinsed to remove residues in 0.2 M HCl then dried for 72 h at 60 °C. Once the tissues reached constant weight, they were ground in a mill until all tissue could pass through a 60-mesh sieve and mixed thoroughly. Tissue N concentration (%) was determined using a NA2500 C/N Analyzer (Thermoquest, CI Instruments LTD, Hindley Green, UK). Tissue P, K, Ca, Mg, Mn, Zn, Cu, Fe, and B concentrations were determined using the dry ash combustion digestion method (Hanlon et al., 1997). A 1.5-g sample of dried ground plant material was weighed and dry ashed at 500 °C for 16 h (Hanlon et al., 1997). The ash was equilibrated with 15 mL of 0.5 M HCl at room temperature for 0.5 h. The solution was decanted into 15-mL plastic disposable tubes and placed in a refrigerator at ≤ 4 °C (Plank, 1992) until analyses by inductively coupled plasma analysis (Munter et al., 1984). Tissue nutrient concentration was compared with critical levels for Florida citrus (Obreza and Morgan, 2008; Obreza et al., 1999).

All sample collection/handling/chemical analysis was done according to standard procedures. A standard curve for certified standards ($R^2 > 0.999$) was developed for each set of samples. Method reagent blanks, standards, and duplicate samples were included for each 10th, 20th, and 30th sample of the tissue samples, respectively.

Tree growth and yield. Tree canopy volume was determined for each tree by measuring the canopy width in a north–south and east–west direction and the tree height. The canopy volume was determined by the formula $V = 0.5236 \times d^2h$ where d = canopy diameter and h = canopy height (Rouse and Wutscher, 1985). Fruit yield was determined by harvesting and weighing all fruit on each tree.

Intraplant CLAs determination. Five to ten leaves from each tree in the trial were collected at four dates: 11 Aug. 2010, 4 May 2011, 21 Dec. 2012, and 3 Feb. 2014. Leaves exhibiting symptoms of HLB (young leaves exhibiting chlorosis patterns similar to Zn deficiency and blotchy mottle chlorosis of older leaves) were chosen for analysis as described by Stansly et al. (2014). Leaves were placed in labeled bags and transported on ice immediately to the SWFREC Huanglongbing Diagnostic Laboratory, Plant Pathology, Immokalee, FL.

Leaf samples were processed using the protocol as described in Stansly et al. (2014)

and real-time polymerase chain reaction (PCR) as described by Li et al. (2006) with a few modifications. Briefly, total plant DNA was extracted from 100 mg of petiole tissue using the Qiagen DNeasy Plant Kit (Qiagen, Valencia, CA). Tissues were lyophilized overnight (16–18 h) before pulverization to a fine powder using a Minibeatbeater (Bio Spec Products Inc., Bartlesville, OK). Samples were then processed as per manufacturer's instruction. Primers and probes were obtained for CLAs [HLBas/HLBr and HLBp (Li et al., 2006)]. Primers and probes for the plant cytochrome oxidase, *COX* gene (COXf/COXr and COX-p) were used for an internal control to control the extraction (Li et al., 2006). The positive control was DNA from previously

tested HLB-positive citrus trees located in the SWFREC grove and negative controls were obtained from citrus grown under psyllid-free screen-house conditions at SWFREC.

Real-time PCR was conducted with an ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) using TaqMan FastUniversal PCRMasterMix (Applied Biosystems) in a 20 μ L volume. The standard amplification protocol was initial denaturation at 95 °C followed by 40 cycles of reactions (95 °C for 3 s, 60 °C for 30 s). Data were analyzed using Applied Biosystems 7500 system SDS software version 1.2. The cycle threshold (Ct) value, is the minimum number of DNA amplification cycles necessary to detect a signal. The sample was

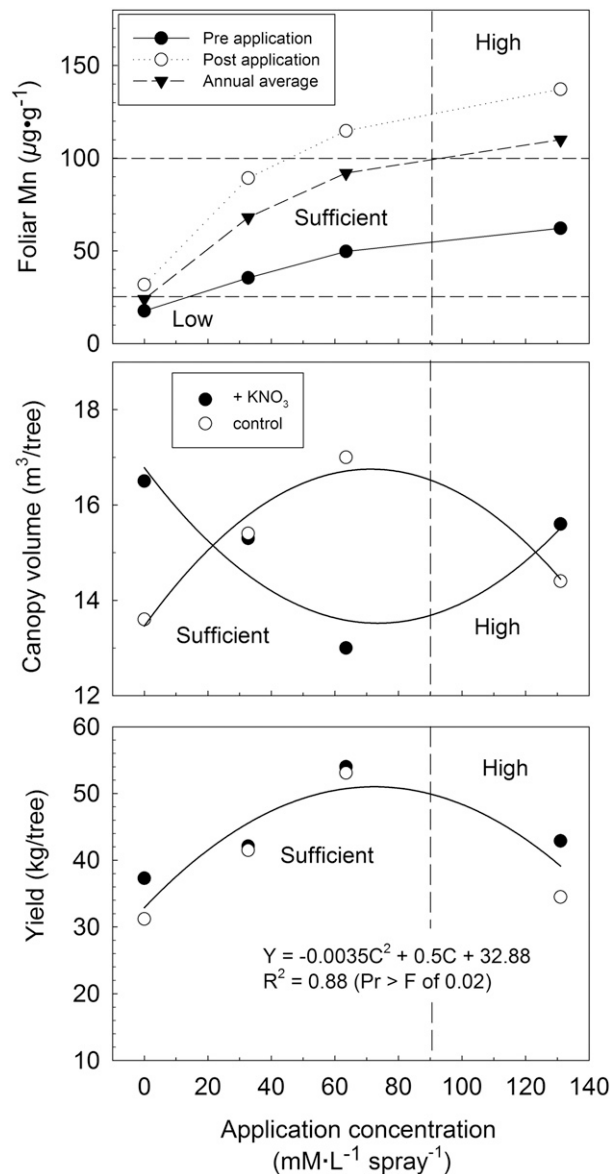


Fig. 1. Foliar concentration of Mn, canopy volume, and yield of ‘Valencia’ trees infected with Huanglongbing (HLB) and treated with different concentrations $MnSO_4$ and with (+ KNO_3) or without (control) foliar applications of KNO_3 . Horizontal lines for foliar Mn delineate the “Low,” “Sufficient,” and “High” ranges that have been considered the traditional levels for trees not infected with HLB. The vertical line delineates the “Sufficient” and “High” ranges of foliar Mn in relation to canopy volume and yield. The x axis is the concentration applied per spray with three sprays per year during each growth flush. The 33, 65, and 131 $mM \cdot L^{-1}$ spray correspond to the 1.5 \times , 3 \times , and 6 \times /year of the recommended rates in Florida, respectively.

considered negative if the Ct value was >36 and positive when the Ct value was ≤36.

CLas vector management. The CLas bacteria associated with HLB is spread by the Asian citrus psyllid (ACP; *Diaphorina citri* kuwayama) which was discovered in 1997 in Florida (Halbert, 1998). Labeled foliar and systemic insecticides were applied to all trees four to five times per year including two dormant season applications according to commercial recommendations using a John Bean Redjet citrus speed sprayer (Durand-Wayland, Inc., Lagrange, GA) whenever ACP were found.

Statistical analysis. Dependent variables were analyzed as a randomized complete block design using the general linear model procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Only interactions of interest among treatments were tested and discussed where appropriate.

Efficacy of uptake of each nutrient applied was determined by testing the interaction of the pre- (1 to 2 weeks before application) and post-measurements (2 to 5 weeks after application) and the various concentrations applied. Since some variables were viewed to exhibit a quadratic response across concentrations, interactions with the concentrations squared were also tested.

Analysis of dependent variables measured annually (foliar concentrations of nutrients tested, canopy volume and yield) were initially tested as split plots over time (year). It would be expected that treatment effects would increase over time and thus justified testing the “year” by treatment interactions. However, the only “year” interaction that was significant was of MnSO₄ for canopy volume. Consequently, the “year” interactions were removed from the rest of the models and “year” was included in the models as a block. When graphed visually, some responses across treatment application concentrations of micronutrients (0×, 1.5×,

3.0×, and 6.0×/year) demonstrated a potential quadratic response so the square of “rate” was included in the model. Where quadratic responses were significant and where appropriate, Sigmaplot (Systat Software, Inc., San Jose, CA) was used to generate a regression and plotted.

Results and Discussion

Grove condition of nontreated plants before and during the study. PCR for CLAs indicated that 83% of the trees tested positive in 2010, 88% in 2011, 99% in 2012, and 98% in 2014. HLB can cause a wide array of decline among trees in a grove so the trees in this experiment were intentionally selected to be in a similar state of decline. Trees not selected for this study demonstrated worse HLB symptoms throughout the entire study. The trees selected for this study were evaluated in 2010 and 2011 for extent of HLB symptoms with 1 = no symptoms and 5 = dead, and the average rating demonstrated intermediate symptoms (average rating = 2.5) that were uniform across trees (rating range = 2.0 to 3.0). Symptoms included interveinal chlorosis of young leaves, chlorotic mottling of older leaves, moderate leaf drop, and some stem dieback. Essential nutrients of foliage for all trees that were not treated in this study were either in the sufficient range or slightly low (Table 2). In particular, P, Cu, and B were in the sufficient range all years whereas N, K, Ca, Mg, Mn, and Zn were low some years and Fe was low all years of the study. Despite some essential nutrients being low in the leaves, the nontreated control trees continued to increase in canopy volume from an average of 11.1 m³/tree in 2011 to 15.5 m³/tree in 2014 and yield from 51 kg/tree in 2010 to 106 kg/tree in 2014.

Expt. 1: KNO₃. The first analysis conducted was to determine whether the foliar application

of KNO₃ affected foliar concentrations of N and K and growth and productivity of the trees. These results will aid interpretation of treatments that evaluated the interaction of micronutrient applications with and without supplemental foliar applications of KNO₃.

The pre- and postmeasurements of foliar N did not demonstrate an increase in leaf N content as indicated by the lack of a significant KNO₃ × time interaction (Pr > F of 0.37) and the lack of a KNO₃ main effect (Table 3; Pr > F of 0.50). The lack of a difference in foliar N for KNO₃ treatments persisted over the 5 years of the study as demonstrated in the annual averages where there was no significant difference in the KNO₃ main effect (Pr > F of 0.95). The annual average foliar N concentration for both treatments was 2.52%, considered optimal for citrus production (Obreza and Morgan, 2008). The lack of an increase in foliar N after application indicates dilution as N was mobilized out of mature leaves and moved to growing shoots and roots, which are synchronized (Bevington and Castle, 1985).

The interaction of KNO₃ treatment of pre- and postmeasurements of foliar K concentration was significant at the Pr > F of 0.19. Foliar K of the controls and KNO₃-treated leaves were 1.02% and 1.24%, respectively. The pre- and postapplication results were supported by the annual mean results where the KNO₃ main effect was significant (Pr > F of 0.02). KNO₃ treatment increased annual foliar K concentration from 1.07% for the controls, which was below the sufficiency range of 1.2% to 1.7%, up to 1.27%, which was within the sufficiency range.

Foliar KNO₃ applications increased canopy volume compared with the controls (Pr > F of 0.06). The average canopy volume of the controls was 13.6 m³/tree, whereas it was 16.5 m³/tree for the controls, representing

Table 5. Response in foliar levels of Zn, growth, and yield of ‘Valencia’ trees with foliar applications of ZnSO₄.

Model	Foliar Zn concn			
	Pre- vs. postapplication of ZnSO ₄ ^z	Annual avg ^y (Pr > F)	Canopy vol (m ³ /tree)	Yield (kg/tree)
Model	<0.01	<0.01	<0.01	0.01
Effects tested in model				
KNO ₃ × Zn rate × time ^x	0.78	—	—	—
KNO ₃ × time	0.88	—	—	—
Zn rate × time	<0.01	—	—	—
Time	0.13	—	—	—
KNO ₃ × Zn rate ²	—	0.88	0.01	0.35
KNO ₃ × Zn rate	—	0.75	0.01	0.27
Zn rate ²	—	<0.01	0.91	0.62
Zn rate	<0.01	<0.01	0.79	0.66
KNO ₃	0.46	0.85	0.13	0.38
Year	—	0.51	<0.01	<0.01
KNO ₃ × block	0.95	0.93	0.22	0.27
Block	0.24	0.24	0.20	0.04
Main effect means				
KNO ₃ treatment				
Control	—	25.4	14.9b	34.8
KNO ₃	—	22.8	15.6a	35.6

The traditional optimum range for foliar Mn is 25 to 100 μg·g⁻¹ dry weight.

^zPre- and postapplication measurements were made between 3 and 7 weeks apart. Pre- and postmeasurements were made twice in 2010 and once in 2011.

^yAverages were made of each treatment and rep for each year and used in the analysis.

^xTime = pre- vs. postapplication of ZnSO₄.

a 21% increase. The $Pr > F$ for the KNO_3 main effect for yield was high ($Pr > F$ of 0.35), but yield for KNO_3 -treated trees was numerically 20% higher than the untreated controls. It is typical that nitrogen and potassium fertilization improve both growth and yield of citrus (Chapman, 1968).

Foliar K was low in control trees. Nevertheless, foliar applications of KNO_3 increased foliar K to well within the sufficient range and along with N-promoted vegetative growth. K deficiency produces a wide array of symptoms in citrus such that no one symptom can be used as a positive identification of its deficiency (Chapman, 1968). Some symptoms are similar to HLB symptoms, including retardation in shoot growth and leaf size, veinal chlorosis and leaf mottling, excessive leaf drop, twig dieback, localized leaf necrosis, and increased fruit drop (Chapman, 1968). K deficiency has been shown to promote loss of turgor and wilting during drought stress and impair stomatal functioning (Lindhauer, 1985), and thus may accentuate plant water deficits in HLB-affected trees where root growth is typically impaired (Graham et al., 2013; Johnson et al., 2013; Ogata, 2011). K deficiency also increases susceptibility of roots to fungal attack (Chapman, 1968) and may promote development of *Phytophthora* root rot of citrus, which occurs more readily in CLAs-infected trees (Graham et al., 2013). Further work needs to be conducted to determine if any of these symptoms are alleviated by foliar applications of K.

Expy. 2: $MnSO_4$ with and without KNO_3 .

The amount of Mn taken up into the leaf was unaffected by KNO_3 as indicated by the nonsignificant $KNO_3 \times Mn \text{ rate} \times \text{time}$ interaction (Table 4; $Pr > F$ of 0.64). $MnSO_4$ treatments demonstrated a significant increase in foliar Mn after application compared with before application as indicated by the significant $Mn \text{ rate} \times \text{time}$ interaction ($Pr > F$ of <0.01). The increase in foliar Mn after application was higher with the higher rate of Mn applied (Fig. 1). The annual average foliar Mn was intermediate between the before and after spray and spanned the spectrum from borderline sufficient/low to slightly high. The annual average varied across application concentrations quadratically ($Rate^2 Pr > F$ of <0.01).

In an initial analysis (analysis and data not shown), the $KNO_3 \times rate^2 \times year$ interaction was included in the model and was significant for canopy volume ($Pr > F$ of <0.01) but not yield ($Pr > F$ of 0.76). The principle difference between the canopy volume curves was at the $131 \text{ mM}\cdot\text{L}^{-1}$ spray ($6\times/year$) treatment where trees that did not receive KNO_3 grew very little, from $14.4 \text{ m}^3/\text{tree}$ in 2011 to $14.9 \text{ m}^3/\text{tree}$ in 2014, whereas the trees that did receive KNO_3 grew from $13.9 \text{ m}^3/\text{tree}$ in 2011 to $18.6 \text{ m}^3/\text{tree}$ in 2014. These data indicate that Mn at the highest rate was excessive when applied without KNO_3 and limited growth; however, growth was restored when N and K were included in the spray.

For ease of presentation and discussion, the $KNO_3 \times rate^2 \times year$ interaction was removed from the model for canopy volume

and the data reanalyzed (Table 4). There was a significant interaction with KNO_3 and the quadratic form of rate of $MnSO_4$ applied ($KNO_3 \times rate^2$) on canopy volume ($Pr > F$ of <0.01). Canopy volume of trees supplemented with KNO_3 was minimum at the $3.0\times/year$ rate of $MnSO_4$ application, whereas trees without supplemental KNO_3 application had a maximum canopy volume at the $3.0\times/year$ rate of $MnSO_4$ application rate (Fig. 1). For both KNO_3 treatments (with and without KNO_3 applied), the $3.0\times/year$ treatment with $MnSO_4$ resulted in a foliar Mn concentration of about $100 \mu\text{g}\cdot\text{g}^{-1}$ dry weight, which is at the top of the range considered sufficient. Unlike canopy volume, the KNO_3

$\times rate^2$ was not significant for yield ($Pr > F$ of 0.39) but yield did demonstrate a significant $rate^2$ effect ($Pr > F$ of <0.01). Yield exhibited a maximum at about the $3.0\times/year$ treatment, which, like the minimum of the canopy volume curve, corresponded with the $3.0\times/year$ treatment of $MnSO_4$. Yield increased from $32.9 \text{ kg}/\text{tree}$ at $0 \text{ mM}\cdot\text{L}^{-1}$ $MnSO_4$ to the maximum of $50.7 \text{ kg}/\text{tree}$ at $65 \text{ mM}\cdot\text{L}^{-1}$, which represents an increase of $\approx 50\%$.

To compare current recommendations for commercial growers for foliar applications of Mn to promote yield of noninfected trees to that found for CLAs-infected trees as in this study, a quadratic regression was developed for the yield curve and used to determine the

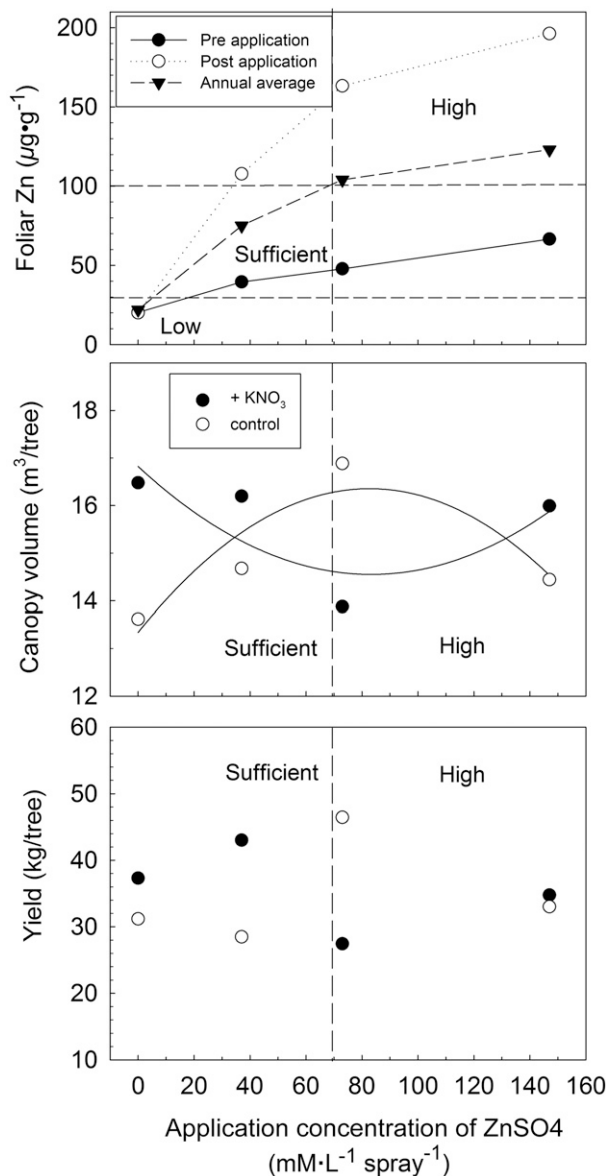


Fig. 2. Foliar concentration of Zn, canopy volume, and yield of 'Valencia' trees infected with Huanglongbing (HLB) and treated with different concentrations and with (+ KNO_3) or without (control) foliar applications of KNO_3 . Horizontal lines for foliar Zn delineate the "Low," "Sufficient," and "High" ranges that have been considered the traditional levels for trees not infected with HLB. The vertical line delineates the "Sufficient" and "High" ranges of foliar Zn in relation to canopy volume and yield. The x axis is the concentration applied per spray with three sprays per year during each growth flush. The 37, 73, and $147 \text{ mM}\cdot\text{L}^{-1}$ spray correspond to the $1.5\times$, $3\times$, and $6\times/year$ of the recommended rates in Florida, respectively.

Table 6. Response in foliar nutrition, growth, and yield of 'Valencia' trees with foliar applications of $Mn_3(PO_3)_2$.

Model	Foliar Mn concn		Canopy vol (m ³ /tree)	Yield (kg/tree)
	Pre- vs. postapplication of $Mn_3(PO_3)_2$ ^z	Annual avg ^y (Pr > F)		
Model	<0.01	<0.01	<0.01	<0.01
Effects tested in model				
Anion × Mn rate × time ^x	<0.01	—	—	—
Anion × time	0.24	—	—	—
Mn rate × time	<0.01	—	—	—
Time	0.06	—	—	—
Anion × Mn rate	—	0.08	—	—
Mn rate ²	—	—	<0.01	0.07
Mn rate	0.20	<0.01	0.03	0.09
Anion	0.35	0.18	0.84	0.07
Anion × block	0.26	0.57	<0.01	0.57
Year	—	0.02	<0.01	<0.01
Block	0.76	0.20	0.01	0.03
Main effect means				
Anion	—	—	—	—
MnSO ₄	—	100	14.6	46.3a
$Mn_3(PO_3)_2$	—	103	15.0	36.9b

The traditional optimum range for foliar Mn has been 25 to 100 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight.

^zPre- and postapplication measurements were made between 3 and 7 weeks apart. Pre- and postmeasurements were made twice in 2010 and once in 2012.

^yAverages were made of each treatment and rep for each year and used in the analysis.

^xTime = pre- vs. post-application of $MnSO_4$ and $Mn_3(PO_3)_2$.

concentration of $MnSO_4$ applied in which yield was within 5% of the maximum. The maximum yield ($Yield_{max}$) was found using the formula $Yield_{max} = c - (b^2/4a)$ and the concentration of $MnSO_4$ applied at which $Yield_{max}$ occurred was determined using $x = -(b/2a)$ where the constants correspond with the curve $Yield_{max} = ax^2 + bx + c$ and x is the concentration of $MnSO_4$ applied ($\text{mm}\cdot\text{L}^{-1}$). $Yield_{max} = 50.7$ kg/tree and 95% of $Yield_{max} = 48.2$ kg/tree. The concentrations of $MnSO_4$ applied that was 95% of $Yield_{max}$ were about 43 and 100 $\text{mm}\cdot\text{L}^{-1}$ spray, respectively, which corresponded to 2.1× – 4.5×/year of the current recommended rate. The 2.1× – 4.5×/year of the current recommended rate occurred where foliar Mn concentration was from about 70–100 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight, which is in the upper end of the 25–100 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight range that has been traditionally recommended for uninfected trees.

Although there was no significant interactions for $KNO_3 \times rate^2$ ($Pr > F$ of 0.39) or $KNO_3 \times rate$ ($Pr > F$ of 0.48) for yield, the KNO_3 main effect was significant ($Pr > F$ of 0.08) with the control and KNO_3 -treated trees producing 40.1 and 44.1 kg/tree, respectively, representing a 10% increase in yield by N and K.

The mechanism of HLB-induced Mn deficiency in citrus is not well established, however, the impairment of fibrous feeder roots before foliar symptom development (Graham et al., 2013) seems to indicate a causal relationship. Feeder roots must be continually produced to explore new regions of soil to extract Mn, which is generally not mobile in most soils. Impairment of feeder root growth by HLB would impede Mn uptake, which would first cause interveinal chlorosis of new leaves because Mn is not mobile in plants. Foliar applications of Mn

alleviate this deficiency (Pustaka et al., 2008; Shen et al., 2013; Stansly et al., 2014). Mn may also play a role in the chlorotic mottling of older leaves where starch build-up has been noted in HLB-affected trees (Achor et al., 2010; Etxeberria et al., 2009; Kim et al., 2009), most likely due to the disruption of phloem loading. Although the specific mechanism of phloem loading that operates in citrus have not been determined, of the three mechanisms that have been proposed to occur in plants, the passive mechanism has been implicated for tree species (Rennie and Turgeon, 2009; Slewinski and Braun, 2010). Passive phloem loading requires Mn as a co-factor for enzymes located in leaf mesophyll cells that produce sugars, which move by osmotic forces into the phloem. The increase in yield with higher concentrations of Mn applied up to 65 $\text{mm}\cdot\text{L}^{-1}$ spray (3.0×/year) supports the conclusion that foliar applications of Mn to infected trees helps promote partitioning of growth toward fruit production, perhaps by promoting more normal phloem loading. However, the 131 $\text{mm}\cdot\text{L}^{-1}$ spray application (6.0×/year) was too high as indicated by a suppression of yield.

The pattern of canopy volume followed that of yield for trees that did not receive KNO_3 , but was concave for trees that were supplemented with KNO_3 . The pattern across application rates for trees that were supplemented with KNO_3 is unusual, but may involve promotion of partitioning of growth away from shoot growth to either root growth or storage. Promotion of root growth with this combination of foliar treatments would be a positive development in understanding approaches to promote overall tree health.

Expt. 3: $ZnSO_4$ with and without supplemental KNO_3 . The lack of a significant $KNO_3 \times rate$ of $ZnSO_4$ applied × time

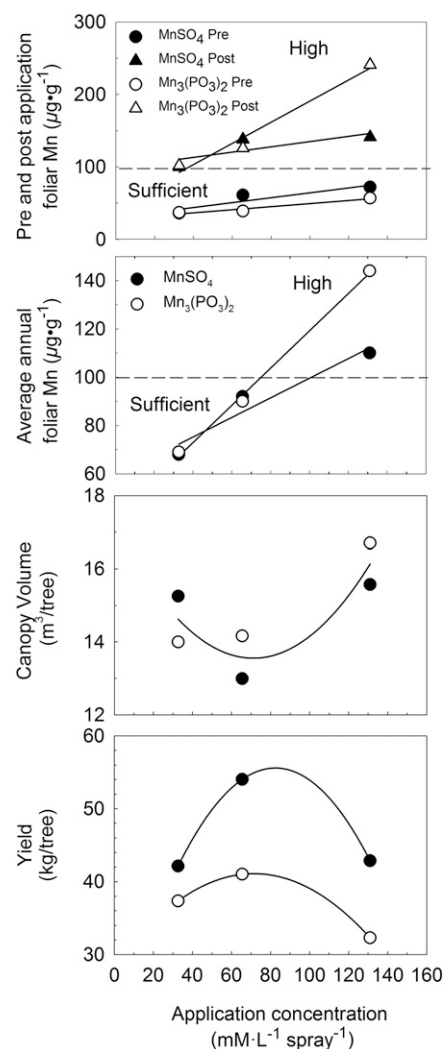


Fig. 3. Comparison of foliar applications of $MnSO_4$ and $Mn_3(PO_3)_2$ on uptake (pre- and postapplication concentrations of foliar Mn), average annual foliar Mn concentration, canopy volume, and yield of 'Valencia' trees infected with Huanglongbing (HLB). Horizontal lines for foliar Mn delineate the "Low," "Sufficient," and "High" ranges that have been considered the traditional levels for trees not infected with HLB. The x axis is the concentration applied per spray with three sprays per year during each growth flush. The 33, 65, and 131 $\text{mm}\cdot\text{L}^{-1}$ spray correspond to the 1.5×, 3×, and 6×/year of the recommended rates in Florida, respectively.

interaction ($Pr > F$ of 0.78) indicates that KNO_3 did not increase or decrease the amount of Zn taken up into the leaf (Table 5). $ZnSO_4$ treatments demonstrated a significant increase in foliar Zn after application compared with before application as indicated by the significant rate × time interaction ($Pr > F$ of <0.01). The increase in foliar Zn after application was higher with the higher rate of Zn applied (Fig. 2). Foliar Zn approached 200 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight for the 6.0×/year application rate, which was almost twice the level of the upper sufficiency range (100 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight). The lack of $KNO_3 \times rate^2$ ($Pr > F$ of 0.88) and $KNO_3 \times rate$ ($Pr > F$ of 0.75) interactions supports the earlier conclusion

Table 7. Response in foliar nutrition, growth, and yield of ‘Valencia’ trees with foliar applications of $Zn_3(PO_3)_2$.

Model	Foliar Zn concn			
	Pre- vs. postapplication	Annual avg ^y	Canopy vol (m ³ /tree)	Yield (kg/tree)
	of $Zn_3(PO_3)_2$ ^z			
		(Pr > F)		
Model	<0.01	<0.01	<0.01	<0.01
Effects tested in model				
Anion × Zn rate × time ^x	0.37	—	—	—
Anion × time	0.46	—	—	—
Zn rate × time	0.19	—	—	—
Time	0.02	—	—	—
Anion × Zn rate ²	—	—	<0.01	0.05
Anion × Zn rate	—	—	<0.01	0.04
Zn rate ²	—	—	0.92	0.28
Zn rate	0.00	<0.01	0.87	0.28
Anion	0.42	<0.01	0.19	0.38
Anion × block	0.48	0.50	0.02	<0.01
Year	—	<0.01	<0.01	<0.01
Block	0.52	0.28	<0.01	0.61
Main effect means				
Anion treatment				
ZnSO ₄	—	105a	15.4	35.1
$Zn_3(PO_3)_2$	—	67b	16.4	34.4

The traditional optimum range for foliar Zn has been 25 to 100 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight.

^zPre- and postapplication measurements were made between 3 and 7 weeks apart. Pre- and postmeasurements were made twice in 2010 and once in 2012.

^yAverages were made of each treatment and rep for each year and used in the analysis.

^xTime = pre- vs. postapplication of ZnSO₄ and $Zn_3(PO_3)_2$.

that KNO₃ does not increase Zn uptake. The annual average foliar Zn concentration was intermediate to the pre- and postmeasurements, responding quadratically across application concentrations as indicated by the significant Zn rate² (Pr > F of <0.01). Annual foliar Zn was 22 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight for control trees and 123 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight for the 6.0×/year treatment. The traditional sufficiency range for Zn is 25 to 100 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight, thus the treatments resulted in leaves that were slightly below the sufficiency range for the controls up to above the sufficiency range for the 6.0×/year treatment.

There was a significant interaction for KNO₃ × rate² of ZnSO₄ applied on canopy volume (Pr > F of 0.01). Trees supplemented with KNO₃ demonstrated a minimum canopy volume, whereas trees without supplemental KNO₃ application demonstrated a maximum canopy volume slightly above the upper limit of the sufficiency range (100 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight), which was a little less than the 3.0×/year treatment. Yield was unaffected by KNO₃ or ZnSO₄ treatment.

The interaction of Zn and KNO₃ on canopy volume was similar to that of Mn applications, which is unusual, but like Mn, may involve promotion of growth away from shoot growth to either root growth or storage. Zinc deficiency symptoms are similar to that of HLB-induced symptoms, including interveinal chlorosis of new leaves; new leaf growth is stunted and internodes are shortened (rosette); stem dieback; and fruit are small, thick-skinned, and misshapen (Chapman, 1968). Severe symptoms result in nearly complete chlorosis of shoots starting at the terminus, similar to the yellow shoot symptoms of HLB. Zn deficiency does not affect yield unless the deficiency is severe

(Chapman, 1968). Foliar Zn were not severely deficient in the current study, thus it would not be expected that Zn applications in this study would impact yield.

Expt. 4: MnSO₄ vs. Mn₃(PO₃)₂. Uptake of Mn varied by the form and the rate it was applied as indicated by the anion × rate × time interaction (Table 6; Pr > F of 0.01). Mn₃(PO₃)₂ increased uptake of Mn in leaves for the highest treatment compared with MnSO₄ (Fig. 3). At the 6×/year treatment, Mn₃(PO₃)₂ increased foliar Mn by 71% compared with MnSO₄. The differences in foliar Mn concentration between the two anions continued with the annual foliar averages where the anion × rate interaction was significant at the Pr > F of 0.08.

There was no significant anion × Mn-rate² or anion × Mn-rate interactions for canopy volume and yield (analysis not shown) so they were removed from the models and the data reanalyzed. Canopy volume varied quadratically across Mn rates of application (Pr > F of <0.01), however, there was no significant anion main effect (Pr > F of 0.84) indicating that the anionic forms of Mn had similar influences on canopy volume.

There was a significant anion × Mn-rate² interaction for yield (Pr > F of 0.07) with a maximum occurring near the 73 mm·L⁻¹ spray (3×/year) treatment. There was a suppression of yield with the PO₃³⁻ anion compared with the SO₄²⁻ anion (Pr > F of 0.07). The average yield of MnSO₄ was 46.3 kg/tree, which was 25% higher than the yield of Mn₃(PO₃)₂, which was 36.9 kg/tree.

The suppression of yield by the PO₃³⁻ anionic compared with the SO₄²⁻ anionic form for Mn²⁺ seems not to be related to a deficiency of PO₄²⁻, which was in the sufficient range for all but the last year of

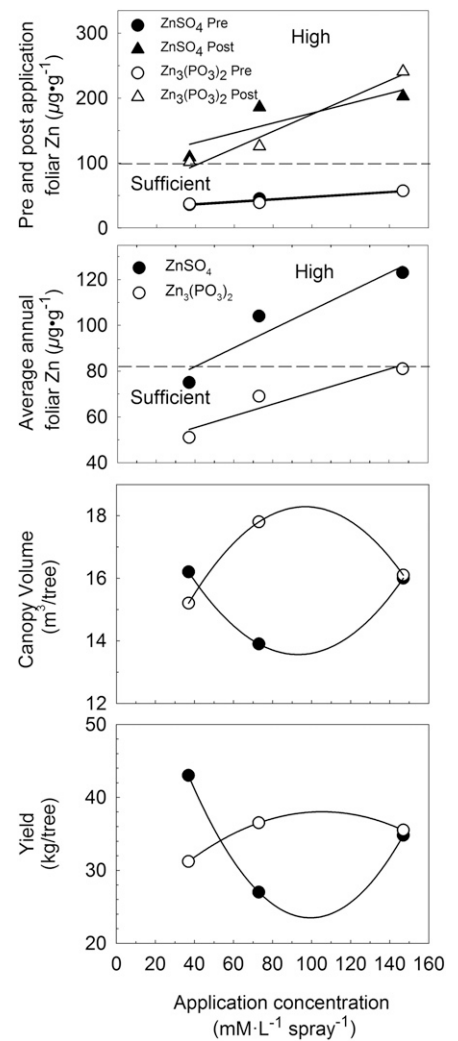


Fig. 4. Comparison of foliar applications of ZnSO₄ and $Zn_3(PO_3)_2$ on uptake (pre- and postapplication concentrations of foliar Zn), average annual foliar Zn concentration, canopy volume, and yield of ‘Valencia’ trees infected with Huanglongbing (HLB). Horizontal lines for foliar Zn delineate the “Low,” “Sufficient,” and “High” ranges that have been considered the traditional levels for trees not infected with HLB. The x axis is the concentration applied per spray with three sprays per year during each growth flush. The 37, 73, and 147 mm·L⁻¹ spray correspond to the 1.5×, 3×, and 6×/year of the recommended rates in Florida, respectively.

this study where it was slightly low at 0.11% (Table 2). However, as discussed previously for Mn where the sufficient range should be reconsidered to be higher than what has been traditionally recommended, it is not known if the foliar P concentrations that are sufficient should be similarly elevated for trees with HLB. If foliar PO₄²⁻ was indeed low, then it is possible that PO₃³⁻ had a negative impact on metabolism (McDonald et al., 2001) and by extension on yield. Furthermore, excess Mn in plant leaves induces oxidative stress (Fernando and Lynch, 2015). Since HLB causes citrus trees to decline, additional stress imposed by other factors such as PO₃³⁻, assuming PO₄²⁻ was truly deficient, and excess Mn,

which was elevated more by increased uptake by the PO_3^{3-} anionic compared with the SO_4^{2-} anionic form, would promote decline.

Expt. 5: ZnSO_4 vs. $\text{Zn}_3(\text{PO}_3)_2$. There was no difference in uptake rate between ZnSO_4 and $\text{Zn}_3(\text{PO}_3)_2$ (Table 7) as indicated by no anion \times Zn rate \times time interaction ($\text{Pr} > F$ of 0.37) or the anion \times time interaction ($\text{Pr} > F$ of 0.46). Unlike $\text{Mn}_3(\text{PO}_3)_2$, the phosphite form of Zn [$\text{Zn}_3(\text{PO}_3)_2$] did not increase foliar concentration of Zn compared with the SO_4^{2-} form (Fig. 4). There was a weak Zn rate \times time interaction ($\text{Pr} > F$ of 0.19) with the higher rates resulting in higher foliar Zn concentration. For the annual foliar Zn analysis, the anion \times Zn-rate interaction was initially tested but was found not to be significant ($\text{Pr} > F$ of 0.31), so was removed from the model and the data reanalyzed. The Zn rate main effect was significant ($\text{Pr} > F$ of <0.01), which was consistent with the pre- and postmeasurements, where the higher application concentrations resulted in higher foliar Zn concentrations. There was also a significant anion main effect ($\text{Pr} > F$ of <0.01) where foliar Zn of the ZnSO_4 treatment was $105 \mu\text{g}\cdot\text{g}^{-1}$ dry weight and the $\text{Zn}_3(\text{PO}_3)_2$ was $67 \mu\text{g}\cdot\text{g}^{-1}$ dry weight. The much lower foliar concentration of Zn for the $\text{Zn}_3(\text{PO}_3)_2$ treatment must have been due to either greater extrusion back out of the leaves or increased mobility into the stems, since the pre- and postmeasurements in uptake were similar for both forms of Zn. Zn has been shown to have limited mobility in plants (Longnecker and Robson, 1993; Palmer and Guerinot, 2009) and, therefore, it is possible that the PO_3^{3-} increased the rate of translocation within the plant.

There were significant interactions in anion \times Zn rate for canopy volume ($\text{Pr} > F$ of <0.01) and yield ($\text{Pr} > F$ of 0.05). The SO_4^{2-} form of Zn resulted in slower growth and smaller canopy volume and yield near the $73 \text{ mm}\cdot\text{L}^{-1}$ spray ($3\times/\text{year}$) treatment whereas the PO_3^{3-} form had the opposite effect. These data indicate that the amount of Zn and the anionic form affect partitioning of growth within the tree.

Expt. 6: Boron. The B rate \times time interaction was significant at the $\text{Pr} > F$ of 0.33 (Table 8) where Foliar B was unaffected at the $0 \text{ mm}\cdot\text{L}^{-1}$ spray ($0\times/\text{year}$) but was about 20% higher at the $22 \text{ (}3\times/\text{year}\text{)}$ and $44 \text{ mm}\cdot\text{L}^{-1}$ spray ($6\times/\text{year}$) treatments (Fig. 5). Although the $\text{Pr} > F$ of 0.33 is high, it is expected that higher concentrations of foliar applications of B would result in increased amounts of B in the leaves. Foliar B concentrations leveled out for the annual measurements such that there was no rate effect ($\text{Pr} > F$ of 0.44 for B rate² and $\text{Pr} > F$ of 0.28 for B rate). The annual average in foliar B was substantially lower than the pre- and postmeasurements. The pre- and postmeasurements were taken the first 2 years of the study, whereas the annual data were collected all 5 years. The lower annual foliar B than the pre- and postmeasurements indicate that foliar B gradually declined throughout the study. The average foliar B was $168 \mu\text{g}\cdot\text{g}^{-1}$ dry weight

Table 8. Response in foliar nutrition, growth, and yield of ‘Valencia’ trees with foliar applications of boron.

	Foliar nutrient concn		Canopy vol (m^3/tree)	Yield (kg/tree)
	Pre- vs. postapplication of B ^z			
	Annual avg ^y ($\text{Pr} > F$)			
Model	<0.01	<0.01	<0.01	0.09
Effects tested in model				
B rate \times time ^x	0.33	—	—	—
Time	0.48	—	—	—
B rate ²	—	0.44	0.01	0.43
B rate	0.24	0.28	0.01	0.59
Year	—	<0.01	<0.01	0.02
Block	<0.01	0.05	0.21	0.22

The traditional optimum range for foliar B is 36 to $100 \mu\text{g}\cdot\text{g}^{-1}$ dry weight.

^zPre- and postapplication measurements were made between 3 and 7 weeks apart. Pre- and postmeasurements were made twice in 2010 and once in 2012.

^yAverages were made of each treatment and rep for each year and used in the analysis.

^xTime = pre- vs. postapplication of BO_3 .

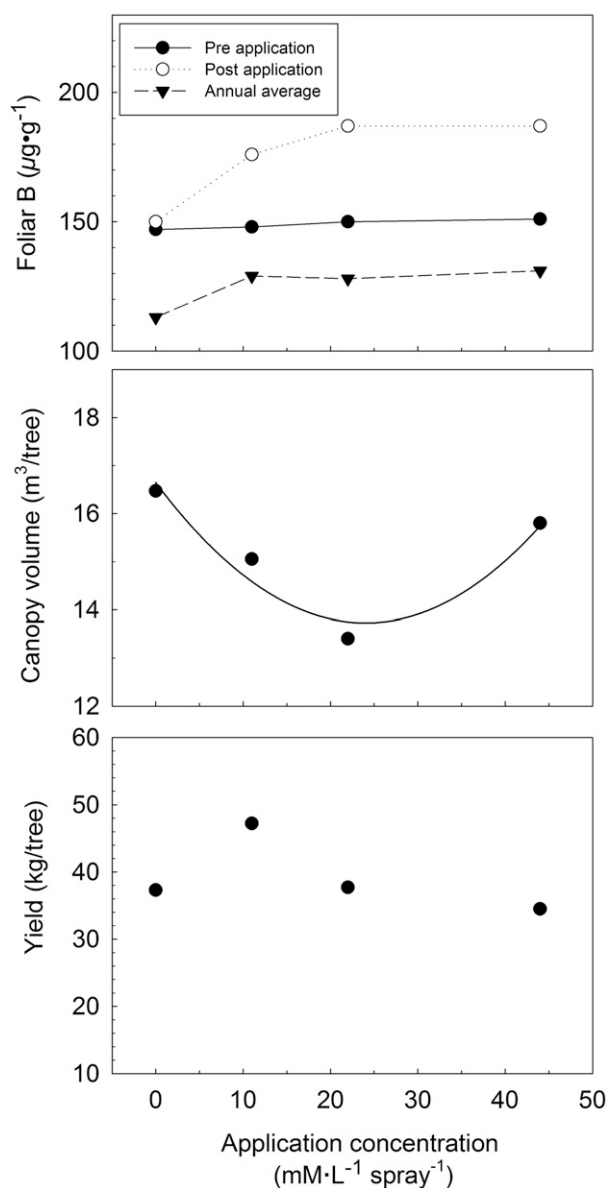


Fig. 5. Boron uptake (pre- and postmeasurements) and annual foliar concentration, canopy volume, and yield of ‘Valencia’ trees infected with Huanglongbing and treated with different concentrations of BO_3 . The 11, 22, and $44 \text{ mm}\cdot\text{L}^{-1}$ spray correspond to the $1.5\times$, $3\times$, and $6\times/\text{year}$ of the recommended rates in Florida, respectively.

Table 9. Response in foliar nutrition, growth, and yield of 'Valencia' trees with foliar applications of MgSO₄.

Model	Foliar nutrient concn		Canopy vol (m ³ /tree)	Yield (kg/tree)
	Pre- vs. postapplication of MgSO ₄ ^z	Annual avg ^y		
	(Pr > F)			
Model	<0.01	<0.01	<0.01	<0.01
Effects tested in model				
Mg rate × time ^x	0.22	—	—	—
Time	0.45	—	—	—
Mg rate ²	—	0.44	0.12	0.28
Mg rate	0.60	0.47	0.08	0.38
Year	—	<0.01	<0.01	<0.01
Block	0.62	0.48	0.08	<0.01

The traditional optimum range for foliar Mg is 0.3% to 0.49%.

^zPre- and postapplication measurements were made between 3 and 7 weeks apart. Pre- and postmeasurements were made twice in 2010 and once in 2012.

^yAverages were made of each treatment and rep for each year and used in the analysis.

^xTime = pre- vs. postapplication of MgSO₄.

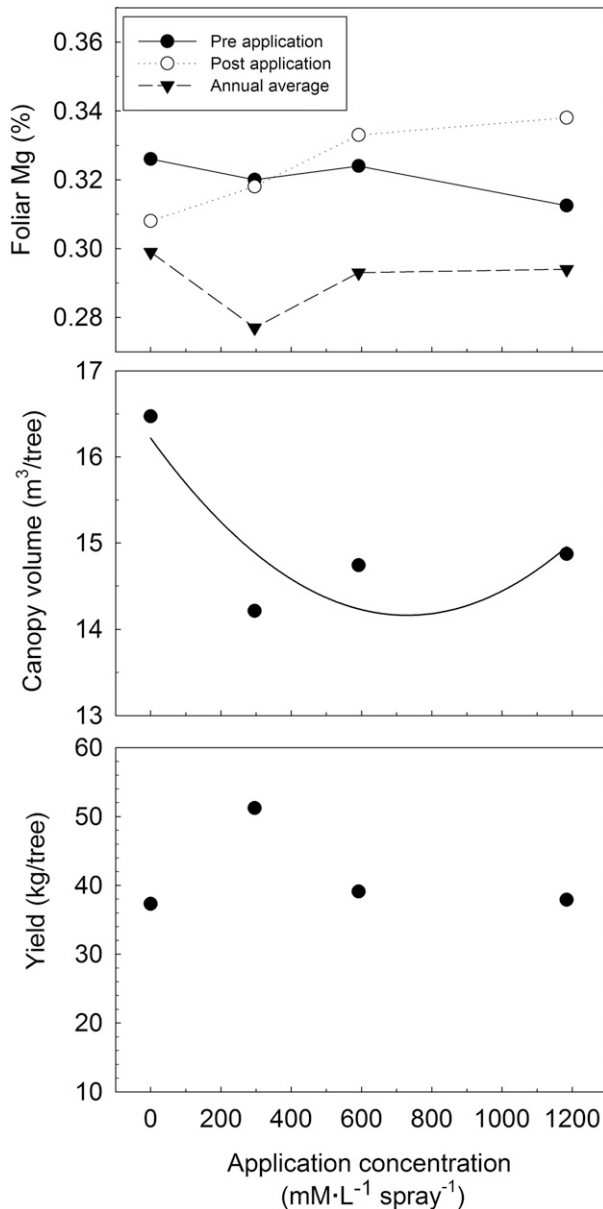


Fig. 6. Mg uptake (pre- and postmeasurements) and annual foliar concentration, canopy volume, and yield of 'Valencia' trees infected with Huanglongbing and treated with different concentrations of MgSO₄. The 296, 592, and 1184 mM·L⁻¹ spray correspond to the 1.5×, 3×, and 6×/year of the recommended rates in Florida, respectively.

in 2010 (considered high leaf concentrations) and declined to 76 μg·g⁻¹ dry weight (within the optimum range) in 2014. This gradual decline also occurred in nontreated trees where the average foliar B was 163 μg·g⁻¹ dry weight in 2010 and declined to 73 μg·g⁻¹ dry weight in 2014 (Table 2).

B is considered to have limited mobility in plants (Shelp, 1993) and when applied to foliage of deficient citrus trees will move from mature leaves to newly developing leaves but not to roots (Liu et al., 2012). B is a critical element necessary for proper root growth (Ali and Jarvis, 1988; Bohnsack and Albert, 1977; Kouchi, 1977). B did not promote shoot growth and in fact shoots grew slowest at the 22 mM·L⁻¹ spray (1×/spray) treatment indicating that mobilization of B was not to promote shoot growth. Foliar B applications also did not affect yield (Pr > F for B rate² of 0.43 and B rate of 0.59) so mobilization of B did not promote yield either.

Expt. 7: MgSO₄. There was a weak Mg rate × time interaction (Pr > F of 0.22) in the pre- vs. post-application of foliar MgSO₄ (Table 9) where the highest rates of application had the greatest increase in foliar Mg (Fig. 6). The annual average was lower and differences in rates of application that existed right after application disappeared such that there was no difference across treatments (Pr > F for Mg rate² of 0.44 and for Mg rate of 0.47). Mg is highly mobile in plants and thus it would be expected that it would move out of leaves to growing points. There was a general decline in foliar Mg from 0.33% in 2010 to 0.20% in 2014 (Table 2). The general decline was also observed in the nontreated plants where foliar Mg declined from 0.34% in 2010 to 0.19% in 2014. The decline in foliar Mg has been observed in HLB-affected citrus that have been fertilized with Mg applied to the root system (Handique et al., 2012).

One of the most important functions of Mg that may have significant implications in HLB-infected citrus is partitioning of photoassimilates throughout the plant. Mg deficiency disrupts partitioning of photoassimilates by the accumulation of nonstructural carbohydrates in leaves (Fischer and Bussler, 1988) and a reduction in transport to other organs of the plant in particular roots where growth can be inhibited (Scott and Robson, 1990). The most probable source of the disruption of partitioning is a suppression of phloem loading (Williams and Hall, 1987). The fate of Mg applied in the current study is unknown, but needs to be determined if it promotes root health for HLB-infected trees.

Summary

Several conclusions can be drawn from this study.

1. Mn with SO₄²⁻ as the balance anion had the largest increase in yield (50% increase from no spray to three sprays per year treatment compared with the control) compared with other macro- and micronutrients applied indicating that it

Literature Cited

- was the most limiting essential nutrient of those tested in this study (Fig. 1). However, yield was 25% lower when applied with phosphite than SO_4^{2-} as the balance anion.
- The foliar concentration of Mn that produced the maximum yield was ($70\text{--}100\ \mu\text{g}\cdot\text{g}^{-1}$ dry weight), which was at the higher end of what has been traditionally recommended for citrus ($25\text{--}100\ \mu\text{g}\cdot\text{g}^{-1}$ dry weight). Maximum yield was achieved by three applications/year of MnSO_4 at $43\text{--}100\ \text{mm}\cdot\text{L}^{-1}$ spray ($2.1\times\text{--}4.5\times/\text{year}$) (Fig. 3).
 - The lack of a yield response of nutrients other than Mn, that is Zn, B, and Mg, does not indicate that they were deficient, but that the low Mn in those trees may have limited their response in this study. The range of Mn of the other treatments was from 9 to $33\ \mu\text{g}\cdot\text{g}^{-1}$ dry weight (Table 2), which was far below the range that produced the maximum yield ($70\text{--}100\ \mu\text{g}\cdot\text{g}^{-1}$ dry weight).
 - KNO_3 treatments had the second most pronounced effect on yield although the effect was more pronounced for canopy volume.
 - There were many concave quadratic relationships in this study that exemplifies the complexity of HLB and nutrition on partitioning of growth. The concave relationships for canopy volume across application concentrations included MnSO_4 and ZnSO_4 without KNO_3 and $\text{Mn}_3(\text{PO}_3)_2$, $\text{Zn}_3(\text{PO}_3)_2$, Boron, and MgSO_4 with KNO_3 . Since the concave relationships were mostly confined to canopy volume and not yield, these results indicate that the partitioning of growth was not toward yield. Considering root decline caused by HLB (Graham et al., 2013), further research needs to be conducted to determine whether the partitioning of growth of the nutrients tested in this study promoted root growth. The minimum points of the quadratic relationships occurred near the traditional $3\times/\text{year}$ recommendation for each nutrient to alleviate deficiency symptoms, although the reason for the minimum occurring at these points is currently unknown.
 - Foliar concentrations of mobile nutrients, in particular N and Mg and to a lesser extent K, did not increase in the leaves indicating that phloem transport of these nutrients was occurring despite the presence of CLAs. This conclusion is supported by the KNO_3 treatments where growth and yield were improved indicating partitioning of those essential nutrients to those growing points. Transport of N, K, and Mg to roots to promote growth, to storage, or to CLAs itself, which likely serves as a sink for essential nutrients may also have occurred but needs to be determined in future studies. Zn and B have limited mobility within plants but the movement of those within HLB-affected citrus is unknown.
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