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Split Application of Essential Nutrients Enhances Tree Growth of HLB-affected Citrus Trees on Florida Sandy Soils

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Studies indicated that greater than 30% of the roots of huanglongbing (HLB)-affected citrus trees are damaged before canopy symptoms appear and that greater than 70% of root loss could be prevalent as citrus deprived of intensive cultural management to reduce abiotic and biotic stresses. The objective of this study was to determine whether split applications of essential nutrients reverse the deterioration of fine root length density (FRLD), tree canopy volume and leaf area index caused by HLB. Statistically significant leaf manganese (Mn) and zinc (Zn) concentrations were detected on the treated trees as compared to the control trees. The results indicated that soil-applied Mn had a positive effect in correcting the nutrient deficiency that might have occurred because of the seasonal crop removal. On the other hand, the inherent bioaccumulation and less mobility of Zn showed that foliar application of Zn could satisfy the annual requirement of Zn by the citrus trees. FRLD and leaf area index (LAI) were significantly affected by the highest micronutrient rate, a manifestation of possible Mn toxicity. These results indicate that citrus trees react positively to the split essential nutrient applications.

Recently, huanglongbing (HLB, or citrus greening) has become the greatest threat to the citrus industry in Florida and worldwide. HLB is a major contributor to citrus yield reduction in Florida (Gottwald et al., 2012; Graham et al., 2020). HLB alters the host plant's physiology, decreases root density by 50%, thus ultimately affecting nutrient absorption, movement, and assimilation (Kadyampakeni et al., 2014; Graham et al., 2013). Poor fibrous density is reflected in weak tree leaf canopy (TCV), deterioration in fruit yield, and poor fruit quality (Morgan et al., 2006; Quiñones et al., 2003). HLB-affected citrus trees with reduced fibrous roots, develop visible symptoms, such as interveinal chlorosis of young leaves, followed by blotchy mottling of older leaves and starch overaccumulation that distorts the grana in the chloroplast (Fan et al., 2013; Hamido et al., 2019). Research has indicated that water uptake by HLB-affected citrus trees is about 18 to 29 % less than by healthy citrus trees (Morgan et al., 2016). Previous studies have reported that trees with lower root density, as occurs in HLB-affected citrus trees, can have lower water uptake and nutrient concentrations (Hamido et al., 2017).

Citrus growers in Florida have been using foliar spraying of micronutrients [manganese, (Mn), zinc, (Zn), and boron (B)] to supply the required nutrients (Gottwald et al., 2012; citrus huanglongbing (HLB, Morgan et al., 2016). Research results have demonstrated mandarin trees grown in various soil types treated with various fertilizer rates have experienced reduced HLB-induced symptoms by approximately 40% (Pustika et al., 2008) These findings supported the assumption that HLB-affected trees have limited soil nutrient uptake and eventually nutrient utilization. Hence, foliar-applied nutrients might extend tree life and increased the yield of HLB-affected trees (Morgan et al., 2016). The rate and method of application of foliar nutrition and their impacts on tree nutrient uptake, accumulation, movement in the soil and the plant are not well understood in HLB-affected citrus trees. Therefore, the objective of this study was to determine whether tree growth, water and nutrient uptake and accumulation in HLB-affected 'Valencia' trees are affected by soil and foliar applied essential nutrients.

Materials and Methods

The study was conducted at the University of Florida, Southwest Florida Research and Education Center (SWFREC) near Immokalee, FL. The study used 15-year-old sweet orange [*Citrus sinensis* (L.) Osbeck] 'Valencia' trees budded on 'Swingle' (Swingle citrumelo [*C. paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] rootstocks. The trees were planted on Immokalee fine sand, which is a poorly drained soil in the Flatwoods, containing sandy marine sediments with slopes < 2%. The experiments were set up in a split-split plot design that contained three nitrogen (N) rates (150, 200, and 250 lb/acre/year) as main block and three soil-applied micronutrient rates including an untreated control at all combinations, with four replications. The N fertilizer was fertigated along with the normal irrigation line in a split on a biweekly basis, summing up to 20 times per year from February to November. The sub-plots had received (1×) [Mn (10 lb/acre/year/), Zn (10 lb/acre/year/), and B (5 lb/acre/year)] treatments randomly within the N rates.

Twenty 4- to 6-month-old leaves were randomly collected across four quadrants (north, south, east, and west) of the canopy of each tree. The leaves were collected from non-fruiting branches located at approximately 2/3 of the height of the canopy. Oven dry samples of 0.5 g dry leaf tissue were weighed and incinerated at 500 °C for 16 h and processed by Inductively Coupled Plasma

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Optical Emission Spectroscopy (ICP-OES) (Spectro Ciros CCD, Fitzburg, MA).

The tree leaf area index (LAI) was estimated using a SunScan canopy device (Dynamax Inc. Huston, TX) during sunny days as per the recommendation of the manufacturer (Kadyampakeni et al., 2014b). Minirhizotron tubes were installed in Mar. 2017 at 0.5 m from either the east or west of the tree trunk (perpendicular to the tree row). Root minirhizotron measurements were conducted on eight trees per treatment (4 micronutrient rates × 4 replications) using transparent minirhizotron acrylic tubes with an external diameter of 2.75 in and 24 in length. The tubes were inserted at an angle of 45° with the respect to the ground and a perpendicular depth of 18 in.

Results and Discussion

Leaf Mn concentration was significantly higher when trees received the foliar (1×) coupled with the soil (1×) or foliar (1×) and the soil (2×) doses treated trees than the control trees. Only the foliar-treated trees showed no significant difference as compared with the control trees, yet within the optimum range of nutrient concentration (Fig. 1A). Leaf Mn concentration had a magnitude of 1.1×, 2.2×, and 3.2× greater for trees under the foliar (1×) only, the foliar (1×) and soil (1×) or foliar (1×) and soil (2×) treatments, respectively. This indicates that the addition 1× more dose to the soil was not worthwhile. In addition, the highest leaf Mn concentration of the highest micronutrient rate was an indication of the highest mobility of Mn from the soil to the tree.

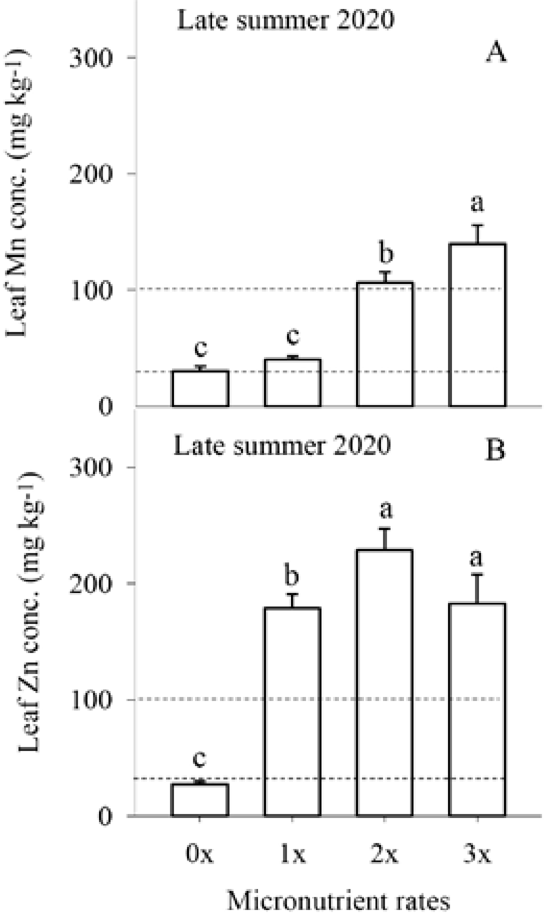

Fig. 1. (**A**) Leaf manganese (Mn) and (**B**) zinc (Zn) concentration on sweet orange [*Citrus sinensis* (L.) Osbeck] 'Valencia' trees budded on 'Swingle' [Swingle citrumelo (*Citrus paradisi* Macf.× *Poncirus trifoliata* (L.) Raf.] rootstocks in reaction to the control (0×), foliar (1×) (1×), foliar (1×) and ground (1×) (2×), and foliar (1×) and ground (2×) (3×), (1× = 10 lb/acre of manganese (Mn) and zinc (Zn) each and 5 lb/acre/year of boron. Error bars indicate the mean values (n = 12 trees) ± standard error of the mean. Different letters on the bars indicate significance at $P < 0.0001$. Horizontal broken lines across the two panels indicate the optimum range of critical nutrient concentrations of leaf Mn and Zn.

All of the treated trees showed excess leaf Zn concentration regardless of the rates and methods of applications (Fig. 1B). Leaf Zn concentration had a magnitude of 2.6×, 5.7×, and 5.4× greater for trees under the foliar (1×) only, the foliar (1×) and soil (1×) or foliar (1×) and soil (2×) treatments, respectively. This indicates that persistent only foliar application can satisfy the annual requirement of Zn to HLB-affected citrus trees. Previous studies also indicated that soil-applied Zn was susceptible to unprecedented soil fixation (Fu et al., 2016) and was less mobile in the trees (Atta et al., 2021b).

There was lower LAI in trees under the highest micronutrient rates, while the other treatments had no significant difference for LAI (Fig. 2). There was 8%, 5%, and –17% of LAI in reaction to the only foliar (1×), the foliar (1×) coupled with the soil (1×) or foliar (1×) and the soil (2×) rate treated trees, respectively. The relative reduction in LAI to the highest micronutrient could be potentially be attributed to Mn toxicity. We also observed the lowest fine root length density (FRLD) when the tree received the highest micronutrient rates.

After the winter season, trees generally started to grow from February onward. Thus, the fine roots simultaneously started to grow accordingly. There was a significant FRLD growth for trees that received foliar or control trees from Feb. to Aug. 2021 (Fig. 3). Since the micronutrients promote above-ground biomass, the FRLD was affected as trees need to balance the above and below-ground growth. However, the highest micronutrient FRLD was significantly lower as compared to the other treatments. This result could be related to the Mn toxicity described earlier. HLB-affected trees react to foliar and ground-applied micronutrient rates such that leaf Mn, Zn concentrations were increased

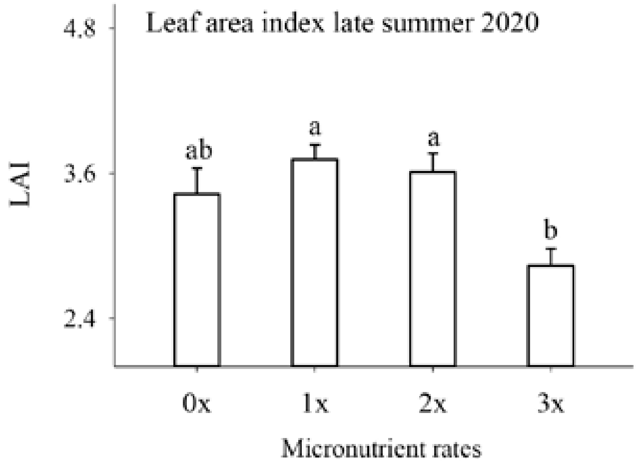

Fig. 2. Leaf area index in sweet orange [*Citrus sinensis* (L.) Osbeck] 'Valencia' trees budded on 'Swingle' [Swingle citrumelo (*Citrus paradisi* Macf.× *Poncirus trifoliata* (L.) Raf.] rootstocks in reaction to micronutrient rates: control (0×), foliar (1×) (1×), foliar (1×) and ground (1×) (2×), and foliar (1×) and ground (2×) (3×), (1× = 10 lb/acre of nanganese and zinc each and 5 lb/acre/year of boron. Error bars indicate the mean values (n = 12 trees) ± standard error of the mean. Different letters on the bars indicate significance at $P < 0.05$.

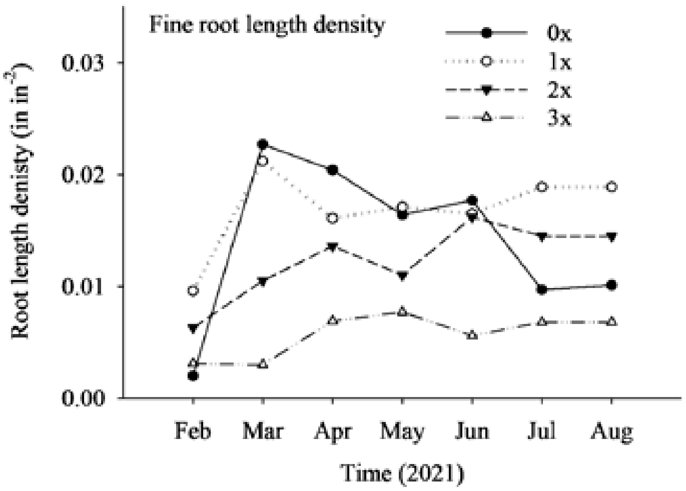

Fig. 3. The effect of micronutrients on fine root length density on sweet orange [*Citrus sinensis* (L.) Osbeck] 'Valencia' trees budded on 'Swingle' (Swingle citrumelo [*Citrus paradisi* Macf.× *Poncirus trifoliata* (L.) Raf.] rootstocks: control (0×), foliar (1×) (1×), foliar (1×) and ground (1×) (2×), and foliar (1×) and ground (2×) (3×), (1× = 10 lb/acre of manganese and zinc each and 5 lb/acre/year of boron on fine root length density during the Spring and Summer 2021 growing seasons.

accordingly. However, micronutrients promoted vegetative growth and compromised FRLD as excess micronutrient was applied to the soil. The highest micronutrient rate (30 lb/acre/year) had a detrimental effect on both above and below-ground tree growth, probably because of Mn toxicity.

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