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Citrus Water Use and Root Density Patterns as Influenced by Citrus Greening and Regulated Deficit Irrigation under Greenhouse Conditions

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Florida citrus production accounts for $8 billion of economic activity and represents about 49% of citrus production in the United States. Current citrus production is at a historical low due to the devastating impact of citrus greening disease (also known as Huanglongbing, HLB) which triggers tree defoliation, root loss, fruit drop and canopy decline. Potential for improving citrus tree performance lies in the ability to develop appropriate water management strategies, good nutrient management options, and the restoration of soil and root health to optimize water and nutrient uptake. A study was conducted in the greenhouse on two-year-old *Citrus sinensis* 'Hamlin' oranges to: 1) determine plant water use under regulated deficit irrigation (RDI) and full irrigation of HLB-affected and non-affected trees; and 2) determine the effect of irrigation rate on tree growth and root density of HLB-affected trees as a function of soil amendments. Results showed that water use was significantly limited for HLB-affected trees grown on sand only, resulting in elevated water contents in the root zone at full irrigation. However, water use was marginally improved when the HLB-affected trees were grown on sand amended with 5% compost and 3% biochar. No significant differences in root density were observed between irrigation and fertilization rates and soil amendments, but significantly lower root densities were noted on HLB positive trees, as expected. Under RDI, canopy size and leaf area were significantly limited for HLB affected compared to non-affected trees suggesting that it would be important to maintain irrigation at optimal level for young trees affected by HLB to avoid compromising tree performance.

Citrus production accounts for $8 billion of economic activity and represents about 60% of total acreage and 49% of production in the United States (USDA, 2018). The production area has declined steadily from 845,260 acres in 1998 to 437,000 acres in 2017, largely due to Huanglongbing (HLB) disease also known as citrus greening (USDA, 2018). HLB has been attributed to decline in tree performance due to reduction in root mass (Graham et al., 2013; Johnson et al., 2013; Kadyampakeni et al., 2014a), reduction in leaf area (Kadyampakeni et al., 2014b), and significant reduction in water and nutrient uptake and biomass accumulation (Kadyampakeni et al., 2014a; Kadyampakeni et al., 2016). In addition, most Florida citrus producing regions are dominated by sandy soils with > 95% sand content and make nutrient and water management extremely difficult (Obreza et al., 2009).

Very little, if any, is known about the interactive performance of HLB- affected citrus trees with respect to water use and soil amendments. However, some recent studies concluded that HLB-affected trees use 22 to 35% less water than non-HLB affected trees (Hamido et al., 2017a), and that frequent irrigation is beneficial for canopy development of HLB-affected trees (Hamido et al., 2017b; Kadyampakeni and Morgan, 2017). Accelerated canopy development coupled with improved water use and nutrient uptake in < three-year-old HLB asymptomatic trees was reported with advanced citrus production systems in central and southwest Florida (Schumann et al., 2009; Kadyampakeni et al., 2016). The work of those researchers (Schumann et al. 2009; Morgan and Kadyampakeni, 2012; Kadyampakeni et al. 2016) did not evaluate the use of soil amendments in improving soil physical properties such as water and nutrient retention and corresponding tree response. In other cropping systems, soil amendments such as compost and biochar have resulted in improved soil properties, including increased interaction of soil microorganisms, fauna and plant roots (Lehmann et al., 2011), improved plant water relations (Basso et al. 2013; Baronti et al. 2014), increased water and nutrient retention, and improved nutrient availability (Hadas et al., 1996; Ozores-Hampton et al.,

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2005). The use of these amendments, in addition to improved understanding and knowledge of the tree growth, root development, and water use would provide the basis for developing appropriate irrigation rates and guide water/nutrient placement for effective water and nutrient use efficiency for HLB-affected citrus in Florida's sandy soils.

In recent years, most citrus producing regions of the world have implemented water saving strategies. The most common strategy is regulated deficit irrigation (RDI), which helps improve water use efficiency and increase crop production while reducing the amount of irrigation water in real-world agriculture (Goodwin and Boland, 2002; Chai et al., 2016). RDI is largely implemented through growth stage-based deficit irrigation or partial root-zone irrigation (Cabra et al. 2008; Goodwin and Boland, 2002; McCarthy et al. 2002; Chai et al., 2016). Nonetheless, there is a lack of understanding of the mechanisms with which citrus trees respond to RDI on Florida sandy soils, and how RDI would influence tree response as a function of HLB.

Water management in Florida's sandy soils requires judicious use of soil moisture sensors when scheduling irrigation. With the sandy soil characteristic (> 95% sand), most manufacturer calibrations of soil sensors are not appropriate and require site-specific calibration for effective interpretation soil moisture data (Morgan et al, 2001, 2002; Deesing et al. 2004; Bandaranayake et al. 2007; Parsons and Bandaranayake, 2007). Thus, the objectives of this study were to: 1) determine citrus water use under regulated deficit irrigation (RDI) and full irrigation of HLB-affected and non-affected trees; and 2) determine the effect of irrigation rate on tree growth and root density of HLB-affected trees as a function of soil amendments.

Materials and Methods

Experimental design. One-hundred forty-four, 12-month-old *Citrus sinensis* 'Hamlin' orange trees on Swingle rootstocks were planted in 5-L buckets. A total of 72 trees were infected with HLB by grafting five leaves on each of the trees with leaves carrying the HLB causing inoculum. One-third of the buckets (48) were filled with 7.1 kg Candler fine sand and 0.4 kg compost (5% by volume), another 48 buckets were filled with 7.3 kg Candler fine sand and 0.2 kg biochar (3% by volume), and the remaining 48 buckets were filled with 7.5 kg sand without amendments. Three nitrogen (N) fertilizer rates included: University of Florida Institute of Food and Agriculture Sciences (UF/IFAS) recommendation for nonbearing trees estimated to be 3.5 mL N/tree/week (Obreza and Morgan, 2008), 75% of UF/IFAS recommendation (2.6 mL N/tree/wk) and 125% of UF/IFAS recommendation (4.4 mL N/tree/wk) with phosphorous (P) and potassium (K) changed proportionally to N. The fertilizer formulation was a growers' liquid fertilizer (Growers Fertilizer Corporation, Lake Alfred, FL) containing 7% N, 0.88% P, 5.8% K 0.1% Mg, 0.01% B, 0.01% copper (Cu), 4% iron (Fe), 0.03% manganese (Mn), and 0.02% zinc (Zn) derived from ammonium nitrate, nitric acid, phosphoric acid, copper sulfate, magnesium nitrate, sodium borate, iron EDTA, manganese sulfate and zinc sulfate. Each fertilization rate had an amendment with: 1) biochar, 2) compost, and 3) no amendment (control). Two irrigation rates, 100% evapotranspiration (ET) and 75% ET, were used. The 75%ET rate is considered the regulated deficit irrigation (RDI). This resulted in a 3 × 3 × 2 factorial experiment replicated four times in a randomized complete block design. This study was conducted for 1.5 years starting in Oct. 2016 and ending in Mar. 2018.

Data Collection

Stem water potential measurements. Stem water potential was measured using 2 leaves per tree at the start of summer in Apr. and May 2017. Leaves were wrapped in plastic and aluminum foil the day prior to data collection to allow the water potential of the leaves to equilibrate with the water potential of the stem. Wrapped leaves were cut at the petiole with a razor blade and stem water potential was measured using a pressure chamber (Model 1000, PMS Instrument Co., Corvallis, OR) that was pressurized at 1 MPa/30 s using compressed nitrogen on a sunny day.

Soil moisture and water uptake measurements. Soil moisture was measured at hourly intervals with HS-10 and EC-5 (Decagon Inc., Pullman, WA) capacitance sensors at 10–15 cm soil depth from 1 Feb. 2017 to 28 Feb. 2018. The capacitance sensors were calibrated gravimetrically at room temperature following earlier methods (Bandaranayake et al. 2007; Parsons and Bandaranayake, 2007).

Average monthly weather data in the greenhouse (temperature, relative humidity, solar radiation, ET_o) were estimated by an automatic weather station (Davis Instruments Corp., Hayward, CA). For citrus ET_c under greenhouse conditions, the study used crop coefficients estimated by Hamido et al. (2017a) for less than three-year-old trees as follows:

$$ET_{cHLB} = K_{cHLB} * ET_{oGH} \quad [1]$$

where:

ET_{cHLB} = citrus evapotranspiration under greenhouse conditions for an HLB-affected tree.

K_{cHLB} = citrus crop coefficient for greenhouse conditions for an HLB-affected tree

ET_{oGH} = potential evapotranspiration under greenhouse conditions estimated with an automated weather station.

$$ET_{cHLY} = K_{cHLY} * ET_{oGH} \quad [2]$$

where:

ET_{cHLY} = citrus evapotranspiration under greenhouse conditions for a healthy citrus tree.

K_{cHLB} = citrus crop coefficient for greenhouse conditions for a healthy citrus tree.

ET_{oGH} = Potential evapotranspiration under greenhouse conditions estimated with an automated weather station.

Tree growth measurements. Tree heights and trunk diameters were measured in March, April, May, August, and September 2017. Trunk diameters were measured in March, April, June, August, and September 2017. Root cores were collected in May and October 2017 using a standard coring method and estimates of root length density were adapted from Kadyampakeni et al. (2014a).

Soil and plant nutrient concentration. Data on soil and plant nutrient concentration were collected but will be reported in a separate publication. Highlights of preliminary results are reported in Kadyampakeni et al. (2017).

Statistical analysis. The data were analyzed using the PROC general linear mixed model procedures in SAS 9.3 Type III (SAS Institute, 2016). Means were separated using Tukey's or Duncan Multiple Range Test procedure. Response variables were soil amendment and irrigation rate on water use, soil moisture and stem water potential. Linear calibration equations for gravimetric vs. sensor based measurements were estimated using regression analysis.

Results

Capacitance sensor calibration. Since the capacitance sensors were calibrated gravimetrically at room temperature following the earlier methods (Bandaranayake et al. 2007; Parsons and Bandaranayake, 2007), good correlations were established between dielectric permittivity and actual soil moisture contents with R^2 of 0.95 to 0.97. The slopes of calibration equations did not differ between amendments but differed between sensors. Capacitance sensor EC-5 showed greater sensitivity than HS10 (Table 1).

Soil moisture distribution between RDI and Full irrigation with respect to soil amendment and HLB. Results showed that water use was severely limited for HLB-affected trees grown on sand only, resulting in elevated water contents in the root zone at full irrigation. Soil moisture in soil without any amendment showed 40 to 52% significantly greater soil moisture than those HLB-affected trees grown on sand amended with 5% compost and 3% biochar. However, no significant differences in soil moisture content were observed between healthy and HLB-affected trees where RDI (75% ET) was used (Fig. 1).

Crop evapotranspiration and sap flow measurements. According to Hamido et al. (2017a), citrus K_c for < 3-year-old trees varies from 0.86 to 1.26 for healthy trees and 0.62 to 0.88 for HLB-affected trees. The study used the monthly K_c estimates provided to determine citrus water use under greenhouse conditions (Fig. 2). For both healthy and HLB-affected trees, monthly crop evapotranspiration (ET_c) was lowest in winter months of December, November, and January, and peaked in July and August (Fig. 2).

Stem water potential and soil moisture relationships. Stem water potential determinations found no significant differences between irrigation rates and soil amendment type, regardless of HLB status (Fig. 3).

The study also attempted to correlate the relationship between SWP and soil moisture content (Table 2). SWP and soil moisture content were negatively correlated on Candler fine sand and sand amended with compost with full irrigation or RDI. Using soil amended with biochar, no clear trend between SWP and soil moisture content was established at full irrigation, and a positive correlation was established between SWP and soil moisture content using RDI (Table 2).

Table 1. Calibration equations for soil moisture sensors HS10 and EC-5.

Soil type	Sensor type	Intercept	Slope (X is the dielectric permittivity)	R^2
Sand only	HS10	–0.1659	0.0003	0.96
Sand + 5% Compost	HS10	–0.1632	0.0003	0.95
Sand + 3% Biochar	HS10	–0.1694	0.0003	0.95
Sand only	EC-5	–0.3436	0.0006	0.97
Sand + 5% Compost	EC-5	–0.3413	0.0006	0.95
Sand + 3% Biochar	EC-5	–0.3017	0.0006	0.96

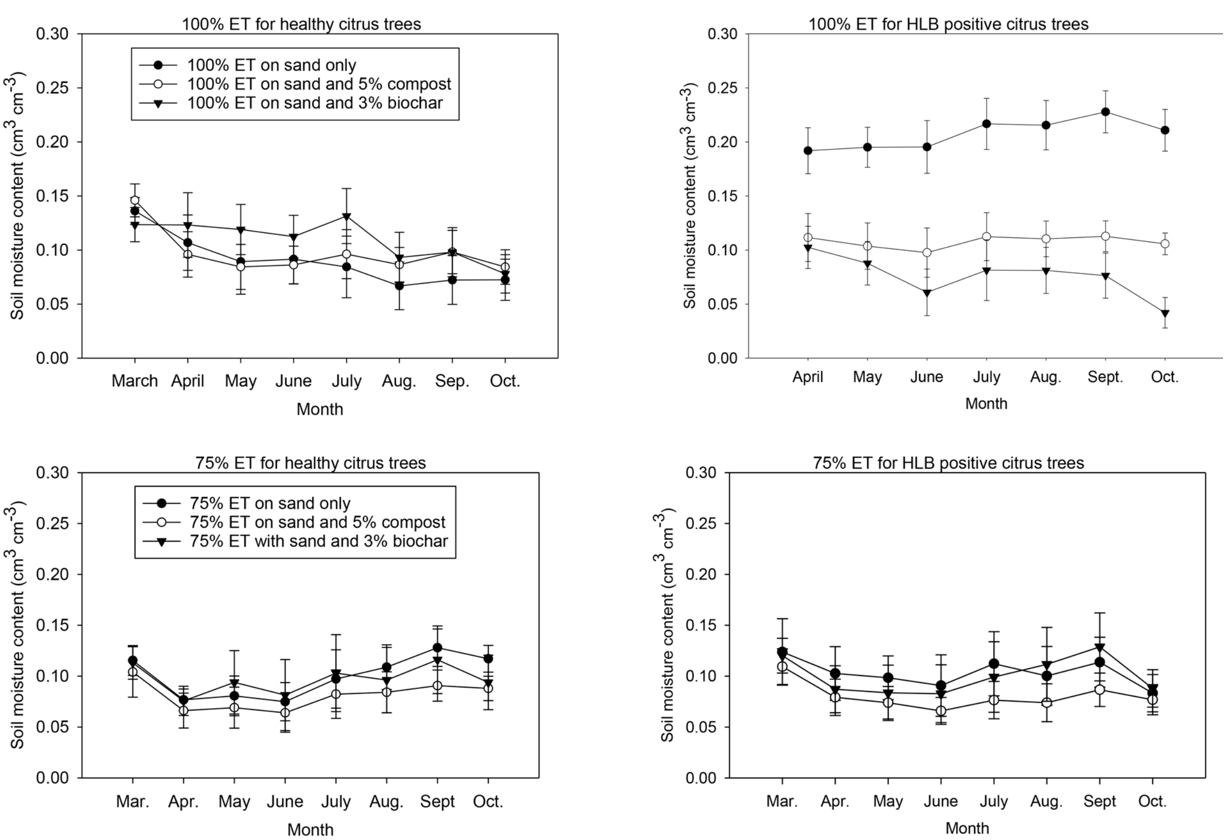

Fig. 1. Temporal soil moisture distribution as a function of citrus greening disease and irrigation rate.

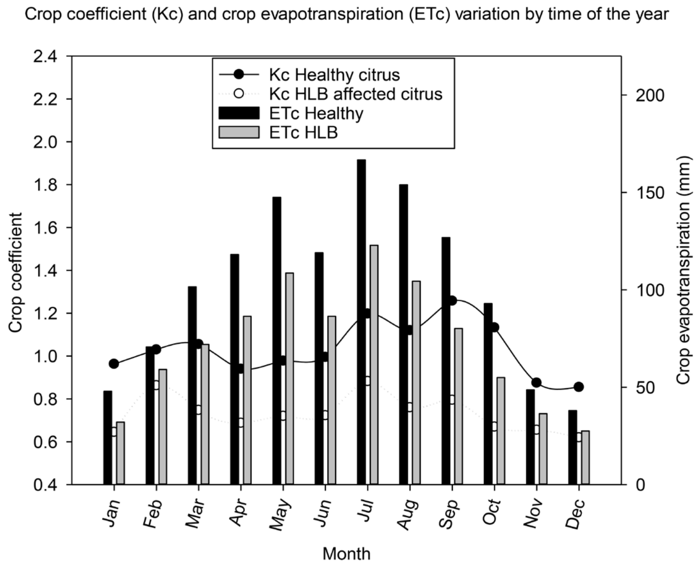

Fig. 2. Crop coefficient and evapotranspiration estimates across the year for 2 to 3-year-old citrus for healthy and HLB affected citrus, Citrus sinensis (source of Kc estimates: Hamido et al., 2017a).

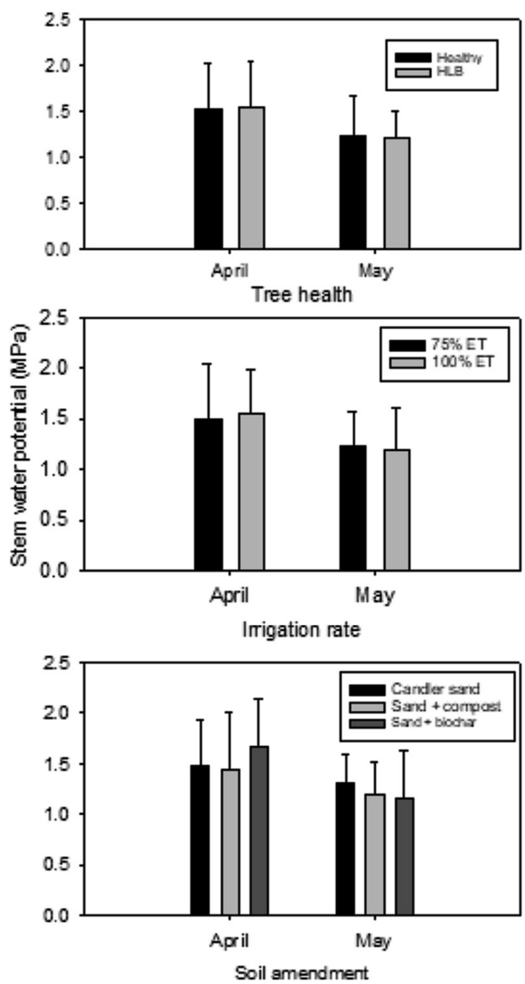

Fig. 3. Water use as a function of *Citrus sinensis* tree health, irrigation rate, and soil amendment. There were no significant ($P < 0.05$) differences in stem water potential as a function HLB status, irrigation rate, and soil amendment.

Table 2. Relating *Citrus sinensis* stem water potential and soil moisture content.

Soil type	Irrigation rate	Intercept	Slope	R^2	RMSE	P
Sand only	Full	2.11	–8.42	0.37	0.35	ns
Sand + 5% Compost	Full	2.23	–9.18	0.42	0.41	*
Sand + 3% Biochar	Full	1.41	0.23	0.00	0.41	ns
Sand only	RDI	3.53	–29.20	0.64	0.47	***
Sand + 5% Compost	RDI	2.44	–10.76	0.26	0.43	ns
Sand + 3% Biochar	RDI	1.88	8.16	0.17	0.46	ns

ns, *,*** Nonsignificant at $P = 0.05$, significant at $P< 0.05$ and $P< 0.001$, respectively.

Tree performance over time. Tree performance did not differ by soil type and HLB status for all the measurement times. However, trunk cross sectional areas (TCA) differed in March ($P = 0.0383$), August ($P = 0.0032$), and September ($P = 0.0002$) (Fig. 4). In March, TCA of the trees irrigated with RDI were 6.3% greater than receiving at full irrigation. In August and September, trees receiving full irrigation showed TCAs 7.2% and 12.4% greater than those under RDI, respectively.

Tree heights were similar by soil type and HLB status at all measurement times, but differed by irrigation rate in August ($P = 0.0231$) and September ($P = 0.0002$) 2017 where trees receiving full irrigation were 5.9% and 14.5% greater in height than those under RDI (Fig. 5).

Root length density (RLD) was not affected by HLB status and irrigation rate, but was affected by soil amendment in May 2017. There was a weak interaction between HLB status and soil amendment and irrigation rate. Seven months later, greater RLD was observed in heathy trees and also where full irrigation was applied (Table 3).

Table 3. Root length density of *Citrus sinensis* as a function citrus greening (HLB) status, soil amendment, and irrigation rate.

Parameter	Root length density	
	1 May 2017	17 Oct. 2017
	---------------- $cm \cdot cm^{-3}$ ----------------	
HLB status	--	--
HLB positive	0.065a[z]	0.184b
Healthy	0.062a	0.247a
Soil amendment		
Sand	0.072a	0.235a
Sand + compost	0.057b	0.213a
Sand + biochar	0.061b	0.198a
Irrigation rate	--	--
RDI	0.063a	0.181b
Full irrigation	0.064a	0.250a
	--------------- ANOVA---------------	
HLB status (HLB)	ns	**
Irrigation rate (Irrig)	ns	**
Soil amendment (Soil)	**	ns
HLB*Irrig	**	ns
HLB*Soil	**	ns
Irrig*Soil	ns	ns
HLB*Irrig*Soil	ns	ns

[z]Means having the same letter in the same columns and under similar subheading are not significantly different using the Duncan Multiple Range Test at $P = 0.05$.

ns, ** Nonsignificant at $P = 0.05$, significant at $P < 0.01$.

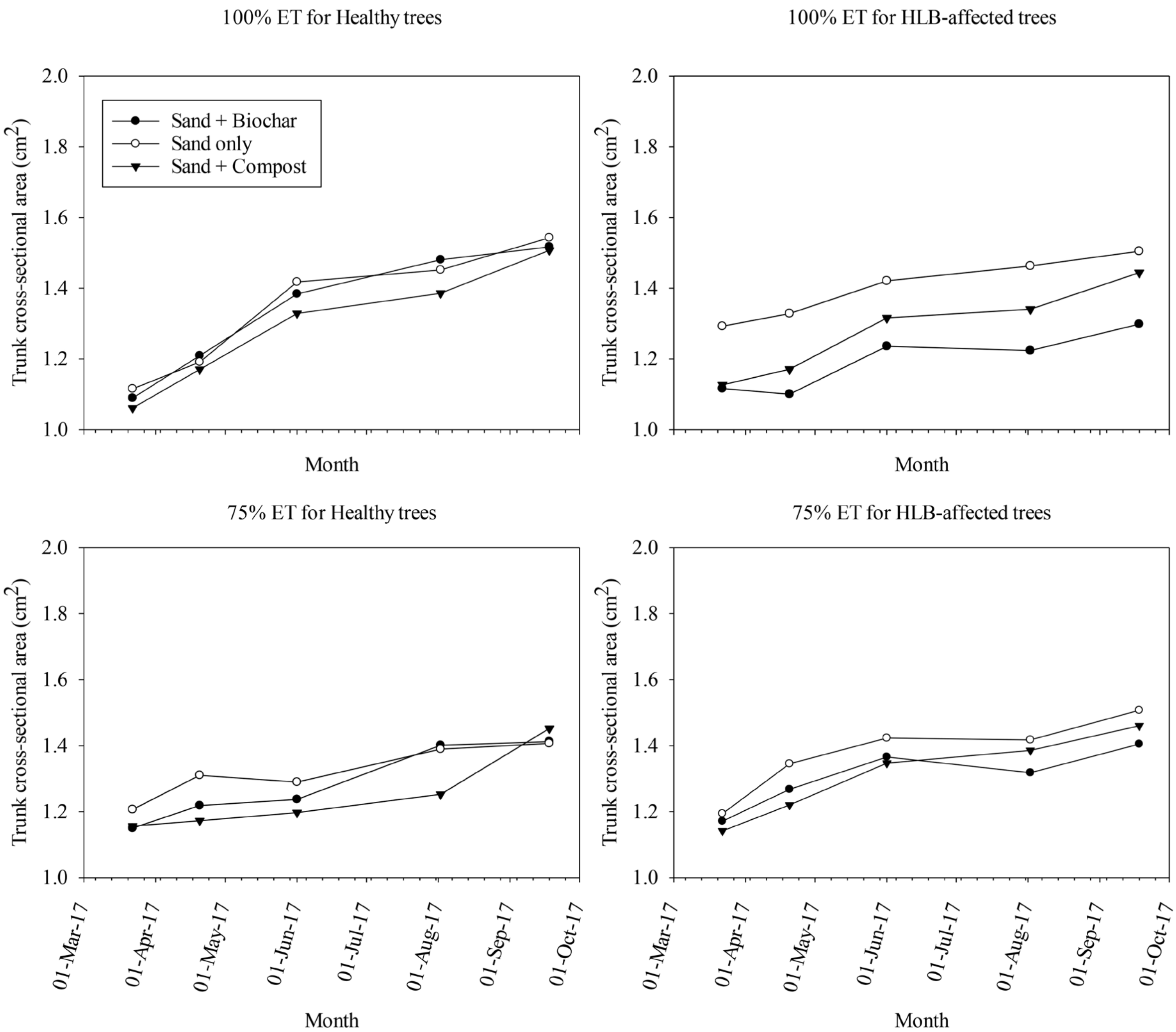

Fig. 4. Mean Citrus sinensis trunk cross sectional area changes over time as function of irrigation rate and soil amendment.

Discussion

Soil moisture content as a function of irrigation rate, HLB and soil amendment. Soil moisture content was not significantly different regardless of the soil amendment and irrigation rate for non-HLB affected trees, and only for HLB-affected trees at full irrigation. Soil moisture data using the calibration equations developed in the study showed that using a pooled or soil specific equation for each type of sensor would still be a more accurate estimate of soil moisture content than the manufacturer's calibration, which might have been determined for soils differing in texture and structure from the sandy soils used in this study. However, for HLB-affected trees, soil moisture content was significantly greater in sandy soils only at full irrigation, suggesting limited tree water uptake compared to trees planted in soils amended with biochar and compost. However, no differences in soil moisture were observed in the soil amendments using RDI, and between HLB-affected and healthy trees.

Water use as a function of irrigation rate, HLB and soil amendment. Stem water potential measurements showed no differences in SWP between healthy and HLB-affected trees, soil amendments, and irrigation rates. The results show that it is plausible to apply 75% of the current irrigation water requirement in Florida, without causing stress in young citrus, thereby helping growers save water. However, correlations of SWP and soil moisture showed no clear relationships in biochar amended soils, indicating that adding biochar would result in greater water repellency and/or hydrophobicity and reduced plant available water as observed by other researchers (Herath et al., 2013; Burrell et al. 2016). Due to the extremely sandy characteristic of Candler fine sand, the authors assumed use of biochar would improve soil physical properties such as water holding capacity. However, it might be important to assess a higher rate of biochar compared to what was used in this study. Candler fine sand and sand amended with compost showed a negative correlation of SWP with soil moisture indicating that improved irrigation management would lower water stress in both HLB-affected and healthy trees. Several researchers showed that frequent irrigation is critical to maintaining good canopy size, leaf area and yield (Hamido et al. 2017a,b; Kadyampakeni and Morgan, 2017). Thus, the irrigation schedule could be updated to accommodate reduced irrigation amounts or irrigation application times (Kadyampakeni et al., 2017). Though no field scale study has been done in Florida, the finding from this study supports the need for improved irrigation management for young, HLB-affected trees using RDI and compost.

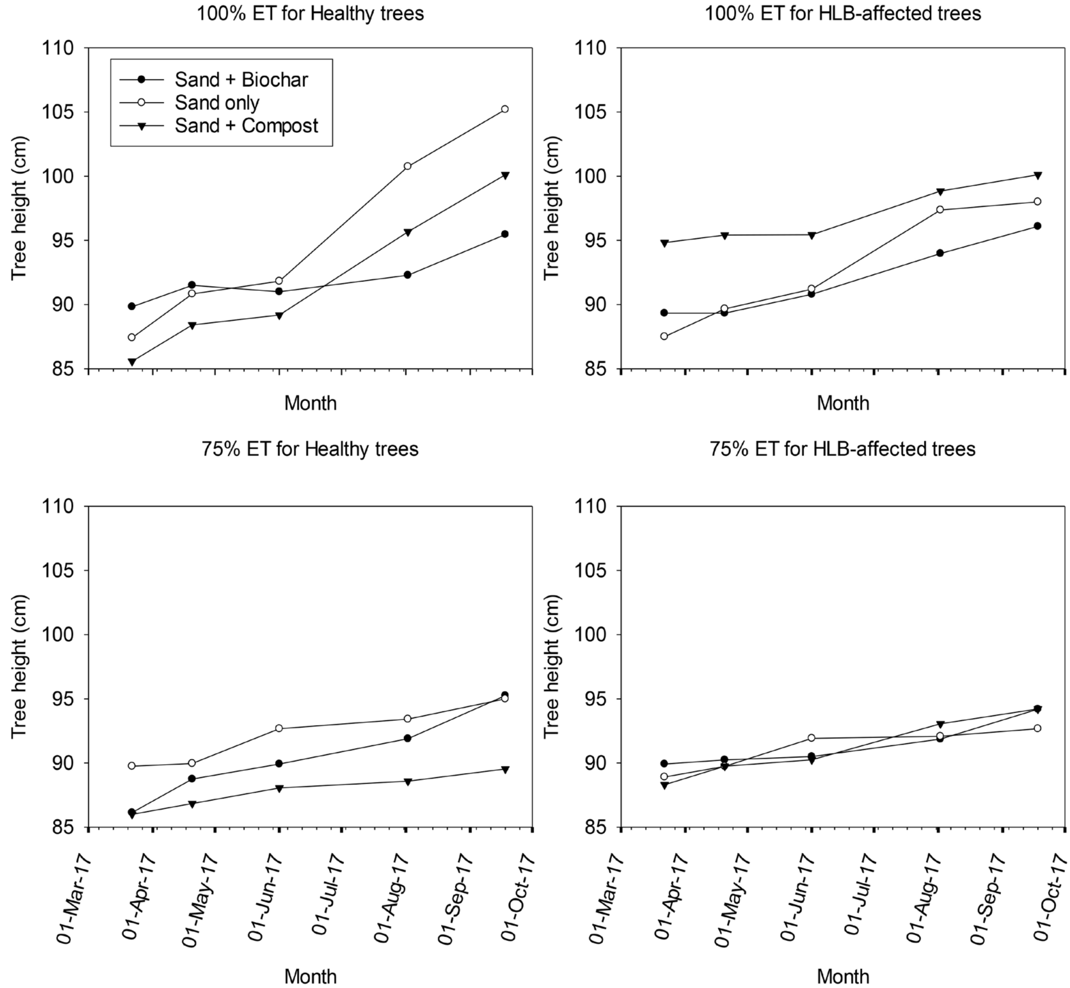

Fig. 5. Mean *Citrus sinensis* tree height measurements as a function of irrigation rate, HLB status, and soil amendment.

Tree performance as a function soil amendment, and irrigation rate. The study showed that HLB status and soil amendment did not limit tree height and trunk development. However, using RDI affected tree performance compared to those trees receiving full irrigation. For example in Mar. 2017, trunk cross-sectional area (TCA) of the trees irrigated with RDI were 6.3% greater than those receiving full irrigation. However, trees receiving full irrigation showed TCAs 7.2% and 12.4% significantly greater than those under RDI in August and September, respectively. These results differ from those of other researchers who reported high tree growth with RDI in olives (Goldhamer, 1999), and peach (Girona et al. 1993).

Though tree heights were similar by soil type and HLB status at all measurement times, results showed that trees which received full irrigation were 5.9% and 14.5% greater in height than those under RDI. Thus, for sandy soils, even with soil amendments, it would be good to maintain the irrigation rate at 100% ET to avoid limiting tree performance. Other researchers reported greater canopy volumes and TCAs with frequent irrigation and fertigation on sandy soils (Kadyampakeni et al. 2016; Hamido et al. 2017b).

Root length density (RLD) showed differences by soil amendment in May 2017 where sand (with no amendment) resulted in 15 to 20% greater RLD than sand amended with either compost or biochar. However, no differences in RLD by soil amendment were observed in October suggesting no benefit of soil amendment on RLD. In Oct. 2017, HLB-affected trees showed 34% lower RLD than healthy trees and those trees receiving full irrigation showed 38% greater RLD than those subjected to RDI. Overall, the study showed that maintaining an optimal irrigation rate is critical to promoting tree vigor and root development. In the study, full irrigation was implemented daily while RDI was applied intermittently, thus, this might explain the need for frequent irrigation to keep the root zone wet or at field capacity most of the time to avoid limiting tree growth and root expansion.

Conclusions

Results showed that water use was limited for HLB-affected trees grown on sand-only, resulting in elevated water contents in the root zone at full irrigation. However, water use was improved when the HLB-affected trees were grown on sand amended with compost but not biochar. Under regulated deficit irrigation (RDI), no differences were observed in water use between HLB-affected and non-affected trees. Though water stress was not observed visually or in stem water potential measurements, RDI limited tree height and trunk cross-sectional area development. Thus for young trees on Florida sandy soils, it would be appropriate to maintain irrigation at full irrigation requirement. In the future, it will be important to assess different application rates of biochar or compost to determine whether a greater rate of biochar or compost would improve tree performance to different water application rates and frequencies on field scale.

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