



Root Density Distribution and Water Uptake of Citrus Trees Infected with Huanglongbing

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Citrus production in Florida is the largest in the United States with a value of production of \$1.34 billion. Yields of bearing citrus trees affected by Huanglongbing (HLB, also known as Citrus greening) (*Candidatus Liberibacter asiaticus*) and infected with Canker (*Xanthomonas axonopodis*) diseases have declined steadily by 30% or more. HLB has been shown to limit root mass that could lower water and nutrient uptake. With such biological threats limiting citrus production, it is important to evaluate root distribution and water use patterns in HLB affected trees to develop appropriate recommendations for optimizing production. Studies conducted on soils in Florida Flatwoods and Ridge growing areas showed that about 64% to 82% of the fibrous roots (< 0.04 inch diameter) of healthy trees were concentrated in the irrigated zones of drip- and microsprinkler-irrigated trees and the rest were found in the non-irrigated zones. The root densities (for roots < 0.02 inch diameter) of non-HLB affected trees at 0–6-inch soil depth at the Ridge site were 1.7- to 4-fold greater than the HLB affected trees at the Flatwoods site in the irrigated and non-irrigated zones, respectively. Mixed results were observed where HLB affected trees under drip irrigation at the Flatwoods site used 8-fold more water per unit canopy volume and 3.4-fold per unit leaf area than the smaller non-HLB affected trees at the Ridge site under the same irrigation system. However, similar water use patterns between HLB and non-HLB affected trees were observed in Summer 2011. The data show that HLB affected and non-HLB affected trees have similar irrigation water requirements as long as the trees have sufficient canopy and leaf mass.

Florida citrus production is the largest in the United States with a value of production of \$1.34 billion. However, the yields of bearing citrus trees affected with HLB disease have declined steadily by more than 30%. HLB is known to reduce root density by 50% or more (Graham et al., 2013; Kadyampakeni et al., 2014a) that could lower water and nutrient uptake. Current citrus fertigation/fertilization practices in Florida are infrequent and low intensity (Alva et al., 2003, 2006; Koo, 1980). The conditions under which intensive fertigation practices work in Florida dominated by well drained sandy soils (Obreza and Collins, 2008) have only recently been investigated (Morgan et al., 2009; Schumann et al., 2009). Intensive fertigation practices are being devised primarily to manage HLB and increase yields so growers break-even within a few years of establishing a grove.

Intensive fertigation practices are already in use for citrus production systems in other countries (Carrasco et al., 2003; Falivene et al., 2005; Kruger et al., 2000a, b; Martinez-Valero and Fernandez, 2004) and have been adapted for Florida sandy soils. A good and detailed understanding of root length density

(RLD) distribution would help in better predicting the patterns of water and nutrient extraction in the citrus root zone thereby guiding the citrus growers and researchers in modifying the current and future irrigation and fertigation practices. However, past root density studies in Florida have concentrated on bearing citrus trees that are older than 5 years (Alva and Syvertsen, 1991; Castle and Krezdorn, 1975; Eissenstat, 1991; Mattos et al., 2003; Morgan et al., 2006, 2007). This study was conducted to 1) determine RLD distribution associated with young trees subject to intensive fertigation management with low volume irrigation and the interaction of HLB on root development; and 2) determine water use in HLB and non-HLB trees.

Materials and Methods

SITE CONDITIONS. The study was conducted at two sites: 1) a site at the University of Florida, Southwest Florida Research and Education Center, Immokalee, Fla. (lat. 26°25'N, long. 81°25'W) with a Spodosol classified as Immokalee fine sand (sandy, siliceous, hyperthermic Arenic Haplaquods) with an excessively drained upper horizon and a low conductivity horizon at less than 3 ft from the surface (Spodosol site, SS); and 2) a site near the Citrus Research and Education Center, Lake Alfred, FL (lat. 28°5'N, long. 81°45'W) with Candler fine sand (hyperthermic, coated Typic Quartzipsamments) an Entisol excessively drained throughout the profile (Entisol site, ES) (Obreza and Collins, 2008). Hamlin orange [*Citrus sinensis* (L.) Osb.] trees on Swingle [*Citrus paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] rootstock

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were planted in April 2006 at 10 ft between trees and 22 ft between tree rows on 44-ft wide beds with drainage swales at SS. Hamlin orange trees on Swingle and C35 [*Citrus trifoliata* L. x *Citrus sinensis* (L.) Osbeck 'Ruby'] rootstocks were planted at 10 x 20 ft and 8 x 18 ft, respectively at ES in December 2008

EXPERIMENTAL DESIGN AND IRRIGATION TREATMENTS. Irrigation and fertilizer treatments were as follows: 1) irrigated with selected allowable soil water depletion between irrigations at the two sites but fertigated monthly with 360° microsprinklers (full-circle microsprinkler) wetting a circle 15 ft in diameter at SS and fertilized with soluble materials 4 to 6 times a year at ES (Conventional); 2) irrigated daily and fertigated weekly with microsprinkler emitters wetting a pattern 3-ft wide and 9-ft long (Restricted Microsprinkler); and 3) irrigated and fertigated daily with drip emitters in short pulses (Drip). The SS had one drip line in the first year of the study and two drippers placed on either side of the tree spaced at 6- and 12-inch from the tree, within the tree row. A second drip line with two drippers placed on either side of the tree was added in the second year of the study. The two drip lines were spaced 12 inch from the tree row. At ES, one drip line was placed within the tree row, with one dripper placed at 6 inch on each side of the tree. All the treatments were laid in a randomized complete-block design replicated four times.

ROOT SAMPLING. Root samples were collected at 0–6- and 6–12-inch depths because most roots of young citrus trees (\leq three-years-old) are concentrated within 12 inches of the soil surface (Fares and Alva, 2000; Paramasivam et al., 2000; Parsons and Morgan, 2004). Root samples at SS were collected in a grid in June 2009 with four samples taken at 6-inch increments in the tree row and three samples taken at 6-inch increments between trees starting at the tree, in one quadrant at two depths at SS. The samples at ES were collected at 4 positions in Dec. 2009 in a 6 x 6 inch grid in a single quadrant at 0–6, and 6–12-inch soil depths. The root sampling method used in the study provides root length estimate in units such as in/in³ of soil volume based on volume of the auger (Böhm, 1979; Escamilla et al., 1991; Lopez-Zamora et al., 2002).

Roots were removed from the soil using a 0.079-inch diameter sieve and hydrated for 15 min. Hydrated roots were separated them into 4 groups: < 0.02 inch, 0.02–0.04 inch, 0.04–0.12 inch and > 0.12 inch using copper wires of known diameter (Kimura and Yamasaki, 1999) before root length determination on a 0.4 x 0.4-inch grid using the line intersection method (Morgan et al., 2007; Tennant, 1975). RLD for each root category was estimated by multiplying the number of horizontal and vertical root intersections by 11/14 and divided by volume of the auger (Mattos, 2000).

ESTIMATION OF STEM FLOW AND EXPERIMENTAL DESIGN FOR WATER USE STUDIES. A randomized complete-block design consisted of three treatments (Drip, Conventional, Restricted Microsprinkler) (Drip, Conventional, Restricted Microsprinkler) at both sites. Plots consisted of three to four adjacent trees serving as replications and a border tree at each end. The irrigation treatments were applied to the replicate trees independently within a row. Water uptake was measured using sap flow sensors (Dynamax Inc., Houston, TX) on one branch each of four trees per treatment (each tree serving as a replicate) at SS from 16 Feb. 2011 to 3 Mar. 2011 and from 3 to 21 June 2011. At the ES, due to limitation in the size of sensors, sap flow measurements on trunks of six trees (with three trees per irrigation method) were taken on Drip and Conventional from 7 to 29 July 2010. From

10 to 22 Mar. 2011, and 23 Aug. to 6 Sept. 2011, sap flow was measured on four trees of each irrigation method at ES. Prior to installation of the sensors, measurements were taken of branch and trunk diameters. The sap flow sensors were connected to a data logger (CR 1000, Campbell Scientific Inc., Logan, UT, USA) to record data every hour. Flow data obtained from the logger (in g·h⁻¹ ≈ 0.0022 lbs/h) were then converted to water flow per unit leaf area per unit time (kg·m⁻²·s⁻¹ ≈ 0.205 lbs/ft²/s, and to mm/d ≈ 0.039 inch/day). The sap flow measurements were done for >2 weeks because that is standard in using the SHB technique (Ham et al., 1990) and for convenience in conducting the experiments at the two sites using the same equipment.

We adapted the approach for determining sap flow measurements from individual plants from Ham et al. (1990) and Lascano et al. (1992). Mean transpiration, T_1 in lbs/ft²/s (=inch/s), was computed by normalizing the stem flow data on a population per land area basis as:

$$T_1 = \sum \left(\frac{f_i}{x_i} \right) * \frac{LAI}{n} \quad (i = 1, 2, \dots, n) \quad [1]$$

where f_i is measured stem flow, lbs/s, x_i is the leaf area, ft², of plant i , and LAI is the leaf areas index of the plot. The transpiration was converted to inch/day by dividing T_1 by the density of water (62 lbs/ft³) and multiplying with 86,400 s (1 d = 86,400 s).

TREE MEASUREMENTS. Leaf area was determined using a portable leaf area meter (Model LI-3000A LI-COR, Lincoln, NB, USA). Leaf area index (LAI) of each tree for each plot was measured using a SunScan canopy analysis system (Dynamax Inc., Houston, TX) during a sunny day. The LAI measurements were taken in two directions: the northwest-southeast and northeast-southwest directions around a tree and were averaged as an estimate of the tree LAI. A calibration curve relating the total leaf area (LA) and LAI was developed for subsequent seasons (LA = 0.24*LAI, $r^2 = 0.82$ at SS and LA = 0.35*LAI, $r^2 = 84$ at ES). Tree canopy volumes were estimated using the formula for a prolate spheroid: (4/3) (π) (tree height/2) (mean canopy radius), by measuring the canopy width in the east-west and north-south directions and canopy height (Obreza and Rouse, 1993). Trunk diameter was estimated from averaging the diameter in the east-west and north-south directions and then calculating the area using the formula πr^2 , where r is the trunk radius.

STATISTICAL ANALYSIS. The data collected on RLD and water use were analyzed using GLM Mixed Model Type III procedures using SAS 9.3 (SAS Institute, 2011).

Results and Discussion

Comparison of root densities by site

At the Flatwoods site, root density increased with depth in both irrigated and nonirrigated zones across the two years. Approximately 50% to 70% of the fibrous roots (< 0.02 inch) in the irrigated zone were concentrated in the top 0–6 inch soil depth with the remaining fibrous roots in the 6–12-inch soil depth in all treatments (Table 1). However, 51% to 92% of the larger roots (> 0.02 inch diameter) in the irrigated zone were concentrated in 6–12-inch soil depth. In 2009, nearly all the fibrous roots (< 0.02 inch) in the non-irrigated zone were found in the top 0–6-inch layer probably because of the rains in summer that provided adequate moisture in the top layer. Roots with diameters

Table 1. Analysis of variance (ANOVA) with means for root length density distribution as a function of irrigation method, soil depth and distance from the tree at the Flatwoods site in June 2009.

Irrigation method ^z	Soil depth (in)	Root diam (in)							
		< 0.02		0.02–0.04		0.04–0.12		> 0.12	
		IRR ^y	NI	IRR	NI	IRR	NI	IRR	NI
----- Root length density (in/in ³) -----									
Conventional	0–6	0.994	– ^x	0.290	–	0.116	–	0.013	–
	6–12	0.677	–	0.303	–	0.206	–	0.032	–
Drip	0–6	1.323	1.097	0.419	0.400	0.110	0.174	0.006	0.013
	6–12	1.310	0.503	0.471	0.374	0.335	0.277	0.071	0.019
Restricted microsprinkler	0–6	1.084	0.877	0.213	0.226	0.052	0.084	0.006	0.006
	6–12	1.000	0.355	0.394	0.213	0.200	0.097	0.039	0.006

ANOVA

Irrigation method	NS	***	***	NS
Depth	***	NS	***	***
Distance from the tree	***	NS	NS	NS

^zConventional = Conventional microsprinkler practice, Drip = Drip open hydroponics system, Restricted microsprinkler = Microsprinkler open hydroponics system.

^yIRR = Irrigated zone, NI = Non-irrigated zone.

^xFor conventional practices, all the sampled positions were irrigated.

NS = Nonsignificant at $P \leq 0.05$; Significant at $P < 0.001$.

Table 2. Analysis of variance (ANOVA) with means for root length density distribution as a function of irrigation method, soil depth and distance from the tree at the Ridge site in December 2009.

Irrigation method ^z	Soil depth (in)	Root diam (in)							
		< 0.02		0.02–0.04		0.04–0.12		> 0.12	
		IRR ^y	NI	IRR	NI	IRR	NI	IRR	NI
----- Root length density (in/in ³) -----									
Conventional	0–6	0.729	– ^x	0.168	–	0.084	–	0.013	–
	6–12	1.419	–	0.245	–	0.168	–	0.000	–
Drip-Swingle	0–6	5.013	2.606	0.265	0.465	0.187	0.110	0.000	0.000
	6–12	2.626	1.684	0.310	0.394	0.284	0.284	0.019	0.019
Drip-C35	0–6	5.232	2.239	0.284	0.387	0.019	0.200	0.013	0.000
	6–12	2.084	0.497	0.123	0.052	0.065	0.032	0.013	0.000
Restricted microsprinkler	0–6	2.761	2.265	0.510	0.348	0.097	0.077	0.000	0.026
	6–12	0.800	0.703	0.239	0.187	0.258	0.084	0.000	0.000

ANOVA

Irrigation method	**	***	***	NS
Depth	***	NS	***	***
Distance from the tree	***	NS	NS	NS

^zConventional = Conventional microsprinkler practice, Drip-Swingle = Drip open hydroponic system with Hamlins on Swingle rootstock, Drip-C35 = Drip open hydroponic system with Hamlins on C35 rootstock, Restricted microsprinkler = Microsprinkler open hydroponic system.

^yIRR = Irrigated zone, NI = Non-irrigated zone.

^xFor conventional practices, all the sampled positions were irrigated.

NS = Nonsignificant at $P \leq 0.05$; **, *** Significant at $P < 0.01$, and $P < 0.001$, respectively.

between 0.02 and 0.04 inch were concentrated in the 6–12-inch soil depth for Conventional and Drip (and in the 0–6-inch-soil depth for Restricted Microsprinkler in both the irrigated and non-irrigated zones. Woody roots (> 0.12 inch) contributed < 3% of total RLD. These results showed greater root densities for Drip and Restricted Microsprinkler than those reported by Morgan et al. (2007) on two to five-year-old Hamlin and Valencia orange trees because trees in that study received infrequent irrigation and fertilization via conventional practice irrigated by full circle pattern emitters with a 10 ft diameter.

In 2009, 46% to 82% of the fibrous and small roots (< 0.04 inch-diameter) were concentrated in the top 0–6 inch

soil depth for the Drip and Restricted Microsprinkler irrigation systems while only 34% to 41% of such roots were found at this layer in the Conventional treatment ($P \leq 0.001$) at Ridge site (Table 2). As at the Flatwoods site, 0.04–0.12 inch-diameter roots were concentrated in the 6–12-inch layer (60% to 77%) in all irrigation methods. Other researchers (Basso et al., 2003; Nappi et al., 1985) also reported similar results. The greater fibrous RLD in the Ridge site compared to the Flatwoods site confirms that HLB damages fibrous roots on young trees (actually even before aboveground symptoms are apparent in three to four-year-old trees in agreement with Graham et al. (2013).

Table 3. Water use for the Ridge and Flatwoods sites as a function of canopy size and leaf area.

Irrigation method	Site	Month/Year	Water use per	Water use per unit	Water use per
			tree per day	canopy volume	unit leaf area
			lbs/day	lbs/ft ³ /day	lbs/ft ² /day
Conventional	Ridge	March 2011	76.92±1.44a	103.55±14.79a	14.24±6.88a
Drip	Ridge	March 2011	10.74±1.72b	74.75±14.01a	12.34±4.04a
Restricted Microsprinkler	Ridge	March 2011	9.70±4.35b	105.89±57.62a	21.12±14.71a
Conventional	Ridge	August 2011	45.39±17.17a	345.70±162.73a	41.05±23.02a
Drip	Ridge	August 2011	98.76±77.44a	292.75±12.46a	27.76±1.19a
Restricted Microsprinkler	Ridge	August 2011	66.41±13.53a	249.93±218.79a	26.10±22.54a
Drip	Flatwoods	February 2011	34.96±16.42	211.00±90.32	41.53±18.51
Conventional	Flatwoods	June 2011	50.61±18.34b	238.25±59.17a	28.24±4.03a
Drip	Flatwoods	June 2011	118.90±56.58a	351.93±129.25a	34.17±8.78a
Restricted Microsprinkler	Flatwoods	June 2011	55.69±26.52ab	239.81±110.56a	52.68±21.83a

^aMean±1 standard deviation, means followed by the same letter in the same column during a particular month are not significantly different at $P = 0.05$ using Tukey's Test.

Daily water use of huanglongbing infected and non-infected trees

Daily water use per unit canopy volume and leaf area were determined for both sites (Table 3). Mixed results were observed in February/March 2011 where HLB infected trees under drip irrigation at the Flatwoods site used more water by 2.8-fold per unit canopy volume and 3.4-fold per unit leaf area than the smaller non-infected trees at the Ridge site under the same irrigation system. However, similar water use patterns were observed in Summer 2011. Another study conducted parallel to this study showed non-HLB infected trees at the Ridge site had 1.5- to 4-fold greater root density than HLB infected trees at SS (Kadyampakeni et al., 2014a). Graham et al. (2013) also found that HLB infected trees experience a reduction in root mass that should limit water uptake. Thus, less water use at the Flatwoods than the Ridge site was expected. However, the results showed that though trees at the Flatwoods site had less root length density than the Ridge site (Kadyampakeni et al., 2014), they used similar or more water per unit leaf area or canopy volume at the Flatwoods site indicating that water use may not be limited by HLB infection as long as the tree has sufficient leaf mass and canopy volume (Kadyampakeni et al., 2014b).

Conclusions

The current study demonstrated that RLDs in the irrigated zones were greater for intensive irrigation and fertigation managed young citrus trees compared with less intensive conventional grower practices. About 64% to 82% of the fibrous roots were concentrated in the irrigated zones of Drip and Restricted Microsprinkler and 18% to 36% was found in the non-irrigated zones at the Flatwoods site. At the Ridge site, the RLD of fibrous roots (<0.02 inch root diameter) for trees grown with intensively managed irrigation systems were three to seven-fold greater at 0 to 6-inch soil depth than that for conventional irrigation management. There was no difference in water use between HLB infected and non-infected trees suggesting similar irrigation water requirements. Thus, use of intensive irrigation and fertigation management should promote citrus root development, water and nutrient uptake and improved management of HLB infected trees without limiting tree production on Florida's Spodosols and Entisols.

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