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Impact of Deficit Irrigation on Growth and Water Relations of HLB-Affected Citrus Trees under Greenhouse Conditions

Samuel Kwakye [1,2] and Davie M. Kadyampakeni [1,*]

[1] Citrus Research and Education Center, Soil, Water and Ecosystem Sciences Department, Institute of Food and Agriculture Sciences, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, USA; skwakye@ufl.edu or skwakye@sandcountyfoundation.org
[2] Sand County Foundation, 44 East Mifflin Street, Suite 1005, Madison, WI 53703, USA
* Correspondence: dkadyampakeni@ufl.edu; Tel.: +1-86-3956-8843

Abstract: Huanglongbing (HLB) is a citrus disease that affects the growth of the fibrous roots of citrus trees. This means that HLB-affected trees may have reduced root volume and may impact water uptake. A greenhouse study was conducted from October 2019 to July 2021 at the Citrus Research and Education Center (CREC) in Lake Alfred, FL, to evaluate the growth and development of HLB-affected citrus trees under a deficit irrigation system. The objective was to assess the impact of deficit irrigation on tree growth, water availability, stem water potential (SWP), sap flow, and root growth of HLB-affected “cv. Valencia” (*Citrus sinensis* (L.)) orange trees on ‘Kuharske citrange’ rootstock (*Citrus sinensis* (L.) × *Poncirus trifoliata*) using an evapotranspiration (ET)-based irrigation schedule. The study hypothesized that HLB-affected citrus trees require less irrigation water to complete their biological functions than healthy citrus trees because of severe fibrous root loss. A total of 20 potted trees were either HLB-positive or non-HLB-affected, and one-half of the trees were subjected to deficit irrigation (80% ET) and the other half to full irrigation (100% ET). There was no significant difference in tree height in both years between HLB-affected trees irrigated at 80% ET and 100% ET. In general, there was no difference in SWP between the HLB-affected trees subjected to deficit irrigation and full irrigation. At 80% and 100% ET, non-HLB trees had greater sap flow than HLB-affected trees. Sap flow for the periods of March–April and June–July 2021 was comparable between HLB-affected trees at all irrigation rates. Maximum sap flow occurred between 11 and 16 h for HLB-affected trees during the three measurement periods. HLB-affected trees had an average water use of 1.6 mm day^{-1} compared to 2.1 mm day^{-1} for non-HLB trees. Healthy trees (non-HLB) used about 20% more water than HLB-affected trees, equivalent to 0.5 mm day^{-1}. Thus, irrigating at 80% ET may be appropriate for achieving water savings in controlled environments for HLB-affected trees without causing water stress. However, since these results were conducted under greenhouse conditions in pots, a follow-up study is needed to validate these results under on-farm field conditions.

Keywords: *Citrus sinensis* (L.) Osbeck; evapotranspiration (ET); huanglongbing (HLB); sap flow; stem water potential (SWP); water-use efficiency

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1. Introduction

In most parts of the world, alternative methods are under evaluation to improve water-use efficiency as water becomes scarce [1]. Water-use efficiency in this context is the ratio of plant productivity to the amount of water used. Optimal irrigation scheduling, including the use of deficit irrigation management, offers an opportunity to reduce water and energy costs, thus potentially increasing water use and profit margin [2,3]. For citrus production, this means that less water is used to either achieve the same or improve productivity, especially in the era of citrus greening, or huanglongbing (HLB). Huanglongbing is a disease that affects citrus trees of all ages, and it is believed to be caused by the bacteria

Candidatus Liberibacter asiaticus (CLas), which is transmitted by an insect vector called Asian citrus psyllid (*Diaphorina citri*) [4–6].

In Florida, HLB is responsible for over 70% of the decline in citrus production from 2006 to 2018 [7]. The roots of the citrus tree are the first site of bacteria multiplication [8], eventually leading to more than 40% loss of fibrous roots [9–12]. Due to the root loss, an infected tree may not require similar amounts of water as a healthy tree, thereby influencing the tree's water use efficiency [13,14]. In Florida, there have been very few research efforts that have investigated HLB-affected trees and water-use dynamics [9,13]. However, none has compared a deficit irrigation schedule to full irrigation for HLB-affected trees. The latter presents a research opportunity to provide information about the response of HLB-affected trees to a deficit irrigation schedule because accurate estimation of water use and stress dynamics could improve irrigation management in citrus production [11,12,15]. Deficit irrigation practices have resulted in substantial water savings compared to conventional irrigation practices, resulting in higher water use efficiencies [16–18], but these impacts might depend on the type of crop or variety of interest [19].

This study was conducted to investigate the effects of applying deficit irrigation on the growth and water-use dynamics of HLB-affected citrus trees. Information provided in this study may help determine the appropriate time to irrigate HLB-affected citrus trees to maximize water uptake, whether deficit irrigation may be appropriate for HLB-affected trees, and elucidate the effect of climatic conditions on HLB-affected trees under deficit irrigation. In citrus production, climatic factors directly affect citrus water requirements [15,20]. Therefore, for an effective irrigation schedule, climatic factors such as solar radiation, temperature, humidity, wind speed, and soil conditions, including soil moisture content, must be monitored with a high degree of accuracy. This is important because irrigation scheduling must be both technically and economically efficient and feasible [2]. An irrigation system is deemed economical if it either increases yield or reduces operational costs [3]. Therefore, the objective of this study was to assess the water use dynamics and root growth patterns of 2 to 4 year old HLB-affected "cv. Valencia" (*Citrus sinensis* (L.) Osbeck) sweet orange trees on 'Kuharske citrange' rootstock (*Citrus sinensis* (L.) Osbeck, × *Poncirus trifoliata*) using evapotranspiration-based irrigation in Florida. We hypothesized that HLB-affected citrus trees require less irrigation water to complete their biological functions than healthy citrus trees because of severe fibrous root loss.

2. Materials and Methods

2.1. Site Description

This study was conducted in the greenhouse at the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Citrus Research and Education Center (CREC) at Lake Alfred, Florida (Latitude 28°5′37″ N; Longitude 81°43′30″ W) from 2019 to 2021. The study used 2 to 4 year old cv. Valencia (*Citrus sinensis* (L.) Osbeck trees on Kuharske citrange (*Citrus sinensis* (L.) Osbeck, × *Poncirus trifoliata*) root stock. A total of twenty trees, including 10 HLB-affected and 10 healthy trees (hereafter called NHLB), were used. Twenty (20) 76 L-size pots were filled with potting mix, and each pot was planted with a citrus tree. The potting mix had compost, perlite, bark, and vermiculite. A potting mix was used because of the complexity (drainage system) of managing a large volume of sandy soil in lysimeters in a controlled environment. Fertilizer was applied in three splits per year: 135 kg N ha^{-1} of calcium nitrate and diammonium phosphate, 67 kg P ha^{-1} of diammonium phosphate, and 100 kg ha^{-1} of potassium sulfate. Other essential nutrients were applied following recommendations by [21]. Thus, fertilization was done in August and December of 2019; April, August, and December in 2020; and April in 2021.

2.2. Irrigation Water Requirement

Each pot was connected to a drip irrigation system with an emitter rate of 4.5 L per hour and controlled by a timer. To estimate the amount of water for each pot per day, a ten year average of meteorological parameters such as solar radiation, air temperature, and relative humidity was collected from the Florida Automated Weather Network (FAWN) station that was located 350 m from the greenhouse (Table 1). Daily reference evapotranspiration (ET_o) was calculated from FAWN using the Penman–Monteith FAO 56 method as described by [22] using Equation (1). The calculated ET_o was then multiplied with average K_c values for healthy trees according to [13] to estimate the daily crop evapotranspiration (ET_c) according to Equation (2). Each pot was covered with mulch, and irrigation events occurred between 7 h and 8 h to minimize surface evaporation.

$$ET_o = \frac{0.40\Delta\ (R_n - \mathrm{G}) + \Upsilon\ \frac{900}{\mathrm{T}+273}\ \mathrm{u}_2\ (e_s - \mathrm{e}_a)}{\mathrm{D}\ +\ \Upsilon(1 + 0.3u_2)} \quad (1)$$

where ET_o = reference evapotranspiration (mm d^{-1}), R_n = net radiation at the crop surface (MJ m^{-2} d^{-1}), G = soil heat flux density (MJ m^{-2} d^{-1}), T = mean daily air temperature at 2 m height (°C), u_2 = wind speed at 2 m height (m s^{-1}), e_s = saturation vapor pressure (kPa), e_a = actual vapor pressure (kPa), $e_s - e_a$ = saturation vapor pressure deficit (kPa), D = slope vapor pressure curve (kPa $°C^{-1}$), and γ = psychometric constant (kPa $°C^{-1}$).

$$ET_c = K_c \times ETo \quad (2)$$

where ET_c = crop evapotranspiration (mm d^{-1}), K_c = crop coefficient, and ET_o = reference evapotranspiration (mm d^{-1}).

2.3. Experimental Design

The experiment was conducted in a split-plot factorial design in pots. Two irrigation treatments equivalent to 100% evapotranspiration (ET) and 80% ET were the main plots, and HLB-affected and non-HLB-affected (NHLB) trees were randomly assigned as subplots to the main plots. The 100% ET was irrigated with 100% of the irrigation water requirement (IWR). Each ET × tree status (HLB-affected or NHLB) combination was replicated 5 times. The treatment structure is presented in Table 2.

2.4. Meteorological Measurements

An automatic weather station (Davis Pro2, Hayward, CA, USA) was mounted in the greenhouse at a height of 2 m to measure weather parameters, following procedures described in [23]. The average solar radiation, minimum and maximum air temperatures, mean air temperatures, and relative humidity (RH) were calculated from the weather station data. Daily reference evapotranspiration (ET_o) was calculated for the greenhouse using the Hargreaves method as described in Equation (3) [1].

$$ET_o = 0.023(0.408)(T_{mean} + 17.8)(T_{max} - T_{min})^{0.5}\ R_a \quad (3)$$

where T_{max} = maximum air temperature (°C), T_{min} = minimum air temperature (°C), R_a = solar radiation (MJ m^{-2}), and 0.408 is a factor to convert MJ m^{-2} to mm. T_{mean} = the daily mean air temperature at 2 m height (°C).

2.5. Tree Growth Variables

Initial tree height and trunk diameter were measured for each experimental unit before starting irrigation treatment applications. Subsequently, a measuring pole height stick (model 807396 by SOKKIA Corporation, Olathe, KS, USA) and a digital caliper were used to measure tree height and diameter, respectively, every six months at the same location on the trunk until the end of the study. The digital caliper recorded the trunk diameter in the north-south (NS) and east-west (EW) directions of the tree. Tree height and trunk diameter growth were estimated by subtracting the initial measurement before treatment application from subsequent measurements.

Table 1. Estimated monthly citrus water use (ET_c) requirement from a 10 year average reference evapotranspiration (ET_o). Parameters for ET_o estimation were retrieved from the Florida Automated Weather Network (FAWN) station in Lake Alfred, Florida, USA.

Month	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	[1] Avg ET_o	[2] K_c	[3] ET_c	[4] IWR
					----------------------- ET_o mm/month -----------------------									- mm/month -	
Jan	47.2	39.4	47.2	47.2	55.1	39.4	47.2	47.2	47.2	47.2	47.2	46.5	0.9	43	39 ± 4.2
Feb	64	49.8	64	64	64	64	56.9	64	71.1	71.1	64	63.4	0.9	56	50 ± 5.9
Mar	86.6	78.7	94.5	102.4	78.7	86.6	94.5	94.5	94.5	94.5	86.6	90.2	0.8	74	67 ± 7.4
Apr	121.9	121.9	137.2	121.9	106.7	114.3	121.9	121.9	121.9	121.9	114.3	120.5	0.7	80	72 ± 7.5
May	133.9	149.6	157.5	141.7	133.9	141.7	149.6	149.6	149.6	110.2	141.7	141.7	0.8	106	95 ± 12.7
Jun	137.2	152.4	144.8	121.9	129.5	137.2	144.8	137.2	114.3	144.8	137.2	136.5	0.8	112	101 ± 11
Jul	133.9	149.6	141.7	141.7	126	141.7	133.9	157.5	133.9	133.9	141.7	139.6	0.9	131	118 ± 8.7
Aug	133.9	126	133.9	118.1	141.7	141.7	126	126	133.9	133.9	118.1	130.3	0.9	113	102 ± 8.2
Sep	106.7	114.3	114.3	106.7	106.7	106.7	106.7	114.3	106.7	114.3	114.3	110.1	1.1	120	108 ± 4
Oct	94.5	94.5	78.7	78.7	86.6	86.6	86.6	86.6	78.7	86.6	-	85.8	0.9	81	73 ± 5.8
Nov	53.3	53.3	53.3	53.3	53.3	53.3	61	53.3	53.3	61	-	54.9	0.9	47	42 ± 3.2
Dec	39.4	31.5	47.2	39.4	47.2	39.4	47.2	47.2	47.2	39.4	-	42.5	0.9	37	33 ± 5.5

Notes: [1] Avg ET_o is the average reference evapotranspiration from January 2009 to September 2019. [2] K_c is the citrus coefficient as described by Hamido et al. (2017). [3] ET_c is the crop water requirement equivalent to 100% ET. [4] *IWR* is the estimated irrigation water requirement (*IWR*) assuming 90% irrigation efficiency. For pots subjected to 80% *ET*, 80% of the *IWR* estimated for 100% *ET* was calculated.

Table 2. Treatment structure description for the evaluation of citrus water use dynamics for Huanglongbing (HLB)-affected and non-HLB-affected (NHLB) "cv. Valencia" orange trees in Florida.

Treatment	Irrigation Requirement by Crop Evapotranspiration ([1] *ET*, %)	Tree Status
1	100	HLB
2	100	NHLB
3	80	HLB
4	80	NHLB

Notes: [1] *ET* is evapotranspiration.

2.6. Leaf Area Measurement

Initial and final leaf areas were measured using ImageJ, a Java-based image processing program, as described in [24]. Twenty fully expanded leaves were randomly selected from each potted tree and scanned with an HP scanner (HP ScanJet Pro 2500 f1, Palo, CA, USA) and saved as JPEG images. At the time of leaf sampling, a total leaf count for each tree was also performed. The saved JPEG images were then imported into the ImageJ application (https://imagej.nih.gov/ij/download.html, accessed on 14 January 2022), where the leaf area was calculated and averaged. The calculated average leaf area was then multiplied by the total leaf count for each tree to estimate the total leaf area.

2.7. Soil Water Content

Soil water content was measured every 30 min for the duration of the experiment by two pronged capacitance sensors (EC-5, Metergroup, Pulman, WA, USA) connected to an EM-50 data logger (Meter Group, Pulman, WA, USA). The sensors were installed at 15 cm depth from the surface of the planting medium and 10 cm away from the trunk of the tree in 3 of 5 replicate pots for each treatment. The average soil moisture content was calculated from the 3 sensors on each treatment.

2.8. Stem Water Potential (SWP)

The stem water potential was measured using a portable pressure chamber (Model 1505D, PMS Instrument Company, Albany, OR, USA). The measurements were performed using a similar procedure described in [25] for higher plants. Four representative leaves per tree (two trees per treatment) were randomly selected and covered with aluminum foil for 24 h to allow the water potential of the leaves to equilibrate with the water potential of the stem. A sharp razor blade was then used to cut leaf petioles close to the stem and placed into the pressure chamber immediately to avoid any biological and/or physical changes. The chamber was pressurized at 1 Bar/30 s (14.5 PSI) using compressed nitrogen until the discharge of water from the petiole became visible and the pressure was recorded (MPa).

2.9. Water Use Relations

Water use was determined using sap flow measurements taken from 28 August to 2 September 2020, 26 March to 9 April 2021, and 14 June to 1 July 2021 using the stem heat balance method with an automated flow system using trunk heat balance gauges SGA10, SGA13, and SGB16 connected to data loggers from Dynamax (Flow32 CR1000x and CR1000; Dynamax, Houston, TX, USA). Stem diameters for the measurements ranged from about 10.1 mm to 16.6 mm during the study. Silicon grease was used to improve the thermal contact of the gauges to minimize trunk injury. For each treatment, 4 out of 5 trees were used, and the sap flow was measured every 30 min for a minimum of a week. The data from the loggers were then converted to water flow per unit diameter size g h^{-1} cm^{-2}. A 24 h daily water flow was calculated for each measuring period and compared among treatments.

2.10. Root Growth

Root growth was assessed monthly using transparent acrylic minirhizotrons installed in each pot using methods described in [26]. The minirhizotrons were installed either to the east or west of the trunk, along the direction of the drip emitter at a 45° angle and 20 cm away from the tree's trunk to a depth of 50 cm from the pot surface. The CID-600 root imager (CID-Bioscience, Pullman, WA, USA) was then used to scan roots within the visible area ($21 \times 19 = 399$ cm^2) of the minirhizotron to estimate root diameter, length, area, and volume. The results were compared among treatments.

2.11. Data Analysis

The two irrigation rates (ET = 100% and ET = 80%) were considered the main plot factors, with tree status (HLB-affected and NHLB) as the subplot factors. An analysis of variance (ANOVA) using the generalized linear mixed model procedure (PROC GLIMMIX) as implemented in SAS (SAS/STAT 15.1, [27]) was used to analyze all response data. When significant (at $\alpha = 0.05$), a multiple comparison using Tukey's post hoc honest significance difference test was performed. Correlations and linear regression between variables were determined using SigmaPlot software (version 12.3; Systat Software Inc., San Jose, CA, USA). An unstructured covariance model (UN) was chosen as the best fit to model the repeated nature of some parameters based on Akaike's Information Criterion corrected for small sample size (AICC). Response variables measured at the end of the experiment were analyzed based on a complete factorial combination of treatment factors, including irrigation rate and tree status. A visual inspection of residuals [28] indicated no violations of the underlying assumptions.

3. Results

3.1. Meteorological Measurements

The mean air temperature inside the greenhouse varied from 22 °C to 27 °C in 2019, 21 °C to 30 °C in 2020, and 19 °C to 28 °C in 2021 during the experimental period (Table 3). The minimum and maximum humidity were 57% and 74%, respectively, throughout the study period. Solar radiation ranged between 3.4 and 8.1 MJ m^{-2} d^{-1} from 2019 to 2021. The calculated ET_o from the greenhouse weather station was between 1.22 mm d^{-1} for the days with minimum air temperatures and 3.99 mm d^{-1} for days with maximum air temperatures. During the period of this study, crop evapotranspiration (ET_c) was calculated for both HLB-affected and non-HLB (healthy) trees. ET_c values ranging from 1.0 to 4.4 mm d^{-1} were calculated for healthy trees, with an average of 2.1 mm d^{-1}. Similarly, ET_c values ranging from 0.7 to 3.0 mm d^{-1} were calculated for HLB-affected trees with an average of 1.6 mm day^{-1}.

3.2. Tree Height, Trunk Diameter, and Leaf Area

The tree height was significantly different ($p = 0.009$) for both years between treatments. In year one, HLB-affected trees subjected to 100% ET had 5–8% greater height than NHLB trees at 100% and 80% ET, respectively (Figure 1). However, between the HLB-affected trees, there were no significant differences in tree height for 100% or 80% ET. In the second year, NHLB trees subjected to 80% ET had a 5–7% increase in height as compared to HLB-affected trees that were irrigated at 80% and 100% ET (Figure 1). The results on height between the HLB-affected trees were comparable. The HLB-affected trees at both 100% ET and 80% ET had at least a 5% increase in trunk diameter compared to the NHLB-affected trees at 100% ET and 80% ET (Figure 1) at the end of year one. In the second year, the changes observed in trunk diameter were comparable for all trees, irrespective of their HLB status or irrigation rate. The leaf area at the end of the second year was not significantly different among all treatments (Figure 2).

Table 3. Average climatic characteristics from the weather station (Davis pro2, Hayward, CA) in the greenhouse. The data presented are the means ± SE of five replicates.

Month, Year	Maximum Temperature	Minimum Temperature	Mean Temperature	Humidity	Radiation	[1] ET_o	[2] ET_c NHLB	[3] ET_c HLB
		---------- °C ----------		(%)	(MJ m^{-2} d^{-1})		------- (mm d^{-1}) -------	
Oct 2019	27.3 ± 1.2	26.2 ± 1.2	26.7 ± 1.2	73.9 ± 4.3	5.0 ± 0.4	2.2 ± 0.2	2.1 ± 0.1	1.6 ± 0.0
Nov 2019	22.9 ± 1.9	21.9 ± 1.8	22.4 ± 1.9	68.9 ± 0.9	4.5 ± 0.8	1.8 ± 0.5	1.5 ± 0.4	1.1 ± 0.3
Dec 2019	22.0 ± 2.6	21.0 ± 2.4	21.5 ± 2.5	71.7 ± 2.8	3.4 ± 1.6	1.2 ± 0.9	1.0 ± 0.8	0.7 ± 0.6
Jan 2020	21.7 ± 2.7	20.7 ± 2.7	21.2 ± 2.7	64.2 ± 2.5	4.1 ± 1.1	1.5 ± 0.7	1.4 ± 0.5	1.1 ± 0.4
Feb 2020	22.9 ± 1.9	21.8 ± 1.9	22.3 ± 1.9	62.9 ± 3.4	5.1 ± 0.4	2.0 ± 0.4	1.8 ± 0.3	1.5 ± 0.1
Mar 2020	22.7 ± 2.1	21.7 ± 2.0	22.2 ± 2.0	56.7 ± 7.8	6.4 ± 0.6	2.4 ± 0.0	2.0 ± 0.1	1.6 ± 0.0
May 2020	27.9 ± 1.7	26.7 ± 1.6	27.3 ± 1.6	69.1 ± 1.0	6.9 ± 0.9	3.2 ± 0.5	2.4 ± 0.2	1.7 ± 0.1
Jun 2020	28.8 ± 2.2	27.6 ± 2.2	28.2 ± 2.2	69.4 ± 1.2	7.1 ± 1.0	3.3 ± 0.6	2.7 ± 0.4	1.9 ± 0.2
Jul 2020	29.4 ± 2.7	28.2 ± 2.7	28.8 ± 2.7	71.3 ± 2.5	6.7 ± 0.7	3.2 ± 0.5	3.0 ± 0.6	2.0 ± 0.3
Aug 2020	29.7 ± 2.9	28.5 ± 2.9	29.1 ± 2.9	71.5 ± 2.7	6.9 ± 0.9	3.3 ± 0.6	2.9 ± 0.5	2.2 ± 0.4
Sep 2020	30.2 ± 3.2	29 ± 3.20	29.6 ± 3.2	71.6 ± 2.7	8.1 ± 1.7	4.0 ± 1.1	4.4 ± 1.6	3.0 ± 1.0
Jan 2021	19.6 ± 4.3	18.3 ± 4.3	18.9 ± 4.3	67.5 ± 0.1	3.7 ± 1.4	1.5 ± 0.7	1.4 ± 0.5	1.1 ± 0.4
Feb 2021	22.5 ± 2.2	21.3 ± 2.2	21.9 ± 2.2	68.0 ± 0.2	3.9 ± 1.2	1.6 ± 0.6	1.4 ± 0.5	1.2 ± 0.3
Mar 2021	23.6 ± 1.4	22.2 ± 1.6	22.9 ± 1.5	65.1 ± 1.8	4.9 ± 0.5	2.2 ± 0.2	1.8 ± 0.2	1.5 ± 0.1
Apr 2021	25.0 ± 0.5	23.6 ± 0.6	24.3 ± 0.5	64.0 ± 2.6	6.1 ± 0.3	2.8 ± 0.2	1.8 ± 0.2	1.7 ± 0.1
May 2021	27.7 ± 1.5	26.3 ± 1.3	27.0 ± 1.4	60.1 ± 5.4	6.4 ± 0.5	3.1 ± 0.4	2.3 ± 0.1	1.7 ± 0.1
Jun 2021	28.8 ± 2.2	27.6 ± 2.3	28.2 ± 2.2	69.0 ± 0.9	6.0 ± 0.2	2.7 ± 0.2	2.2 ± 0.0	1.6 ± 0.0
Jul 2021	28.4 ± 2.0	27.5 ± 2.1	28.0 ± 2.1	74.2 ± 4.6	6.4 ± 0.6	2.7 ± 0.2	2.5 ± 0.3	1.7 ± 0.1

Notes: [1] ET_o is the reference evapotranspiration. [2] ET_c NHLB is citrus crop water used for healthy trees. [3] ET_c HLB is citrus crop water used for citrus trees with greening (HLB-affected trees).

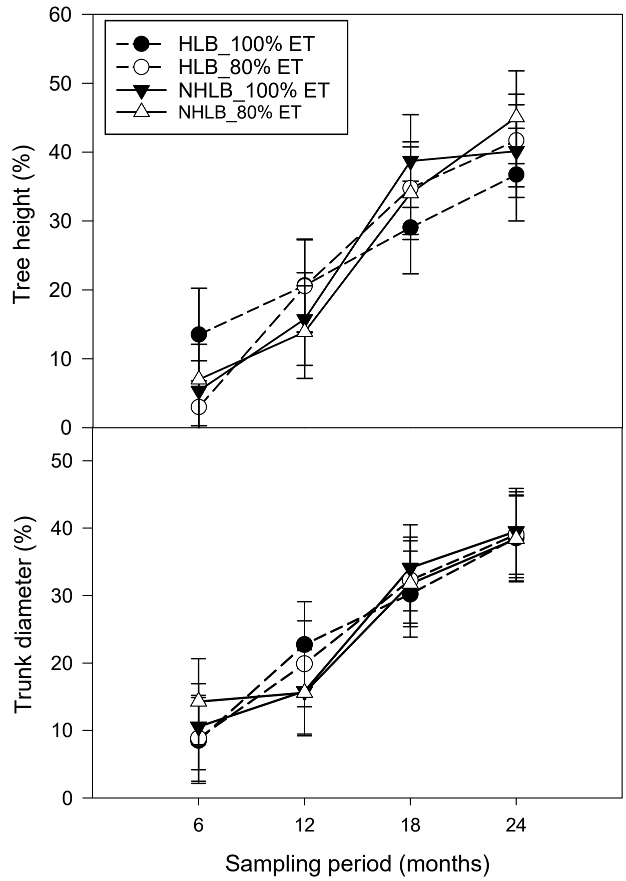

Figure 1. Percent change in tree height and trunk diameter on huanglongbing (HLB)-affected and non-HLB-affected (NHLB) "cv. Valencia" trees at two evapotranspiration (ET)-based irrigation rates (100% and 80% ET) from 2019 to 2021. The percentage change referred to the change between the baseline measurement and the sampling period (monthly) measurements.

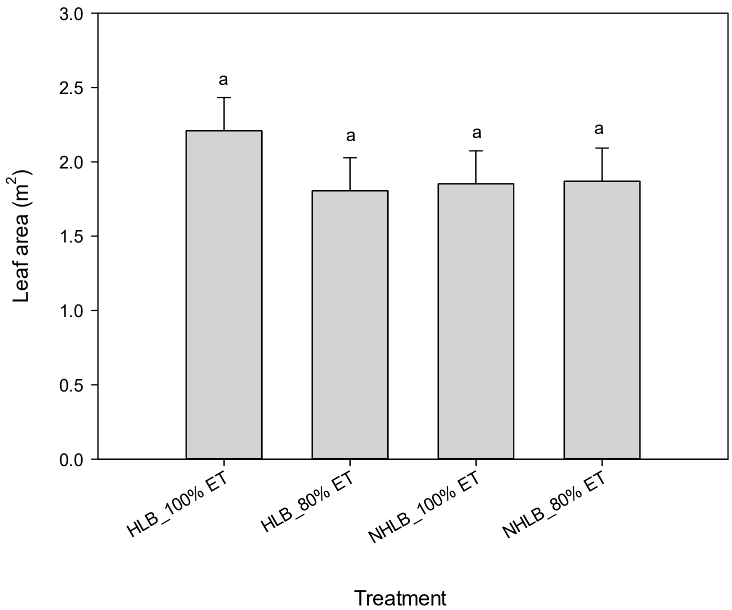

Figure 2. Total leaf area of huanglongbing (HLB)-affected and non-HLB-affected (NHLB) "cv. Valencia" orange trees subjected to two evapotranspiration (ET)-based irrigation rates (100% and 80% ET) in July 2021. The means within a treatment followed by the same letter are not significantly different at $\alpha = 0.05$.

3.3. Soil Water Content

The soil water content in the irrigated zone was between 0.10 and 0.35 cm^3 in all the pots. The trees under 100% ET had the highest values, with about 30% more moisture content than the trees under 80% ET (Figure 3). Generally, soil moisture content peaked moments after irrigation and then dropped and stabilized until the next irrigation schedule as a result of root water uptake.

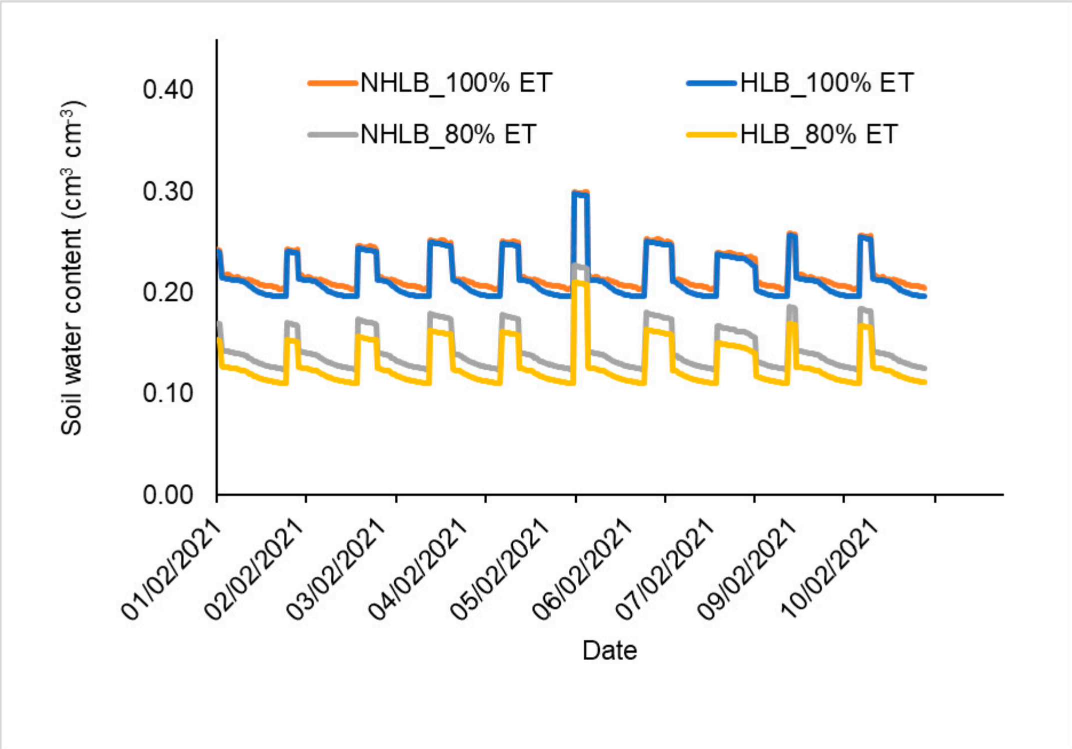

Figure 3. Soil moisture content data at 15 cm depth in the irrigated zone of huanglongbing (HLB)-affected and non-HLB-affected (NHLB) "cv. Valencia" trees under two evapotranspiration (ET)-based irrigation rates (100% and 80% ET) on 1 February to 15 February, 2021.

3.4. Stem Water Potential

Stem water potential (SWP) was significantly different among treatments for August 2019, October 2019, and September 2020, with p-values of 0.0113, 0.002, and 0.0011, respectively. However, there was no significant difference between treatments for the periods of March 2020, May 2020, November 2020, and July 2021. The results for the SWP ranged between −2.4 and −0.6 MPa, similar to the ranges reported by [17,18] using deficit irrigation practices. The lowest values were recorded in May 2020 for all treatments. The HLB-affected trees under both 80% and 100% ET had similar SWPs for all the periods (Figure 4). However, the NHLB trees under 80% and 100% ET showed some variation with respect to SWP (Figure 4).

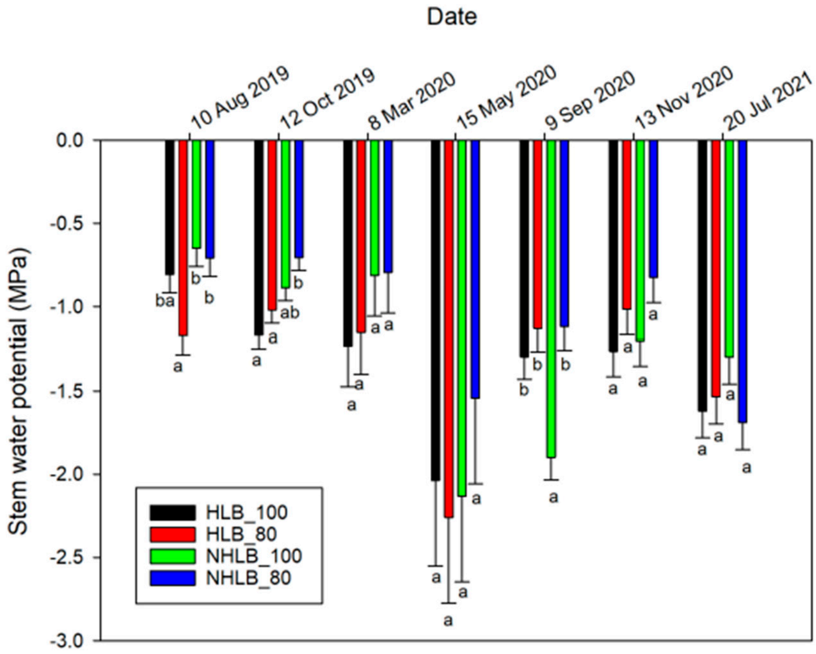

Figure 4. Effect of stem water potential (MPa) on huanglongbing (HLB)-affected and non-HLB-affected (NHLB) "cv. Valencia" trees under two evapotranspiration (ET)-based irrigation rates (100% and 80% ET) from 2019 to 2020. Means within a measurement date followed by the same letter are not significantly different at $\alpha = 0.05$ (Tukey post hoc tests, $p \leq 0.05$).

3.5. Sap Flow Dynamics

In August–September 2020, trees subjected to a deficit irrigation schedule showed a 12% greater sap flow as compared to trees subjected to a full irrigation schedule (Figure 5). However, in March–April 2021, NHLB trees subjected to a full irrigation schedule (100% ET) had 28% greater sap flow than the other treatments. In June–July 2021, NHLB trees subjected to 80% ET had a 30% greater sap flow than all other treatments (Figure 5). Generally, sap flow occurred between 8 and 20 h daily. Sap flow (g h^{-1} cm^{-2}) peaked between 12 h and 14 h, 12 h and 15 h, and 11 h and 12 h per day in August–September 2020, March–April 2021, and June–July 2021, respectively (Figure 5).

A regression analysis of citrus water use was performed on a 24 h average period for HLB-affected and NHLB trees at 80% and 100% ET (Table 4). In August–September 2020, the trees subjected to 80% ET, irrespective of HLB status, showed at least a 40% greater slope when compared to the trees subjected to full irrigation. Thus, these trees had a greater increase in sap flow per hour with time. NHLB trees that were subjected to full irrigation had at least a 94% rise in sap flow as compared to all other treatments in March–April 2021. However, in June–July 2021, NHLB trees subjected to 80% ET had at least a 100% rise in sap flow per hour when compared to other treatments. Trees subjected to 100% ET showed a better fit (with a lower RMSE) to the regression model than trees subjected to 80% ET for the spring and summer measurements, while in the spring, trees subjected to 80% ET showed a better fit to the regression model.

3.6. Root Growth

The root growth was significantly different among treatments. The total root volume and area ranged between 175 and 491 mm^3 and 795 and 1253 m^2, respectively (Figure 6). The root volume for NHLB trees at 80% ET was 50–60% greater than that of HLB-affected trees at 100% and 80% ET, respectively (Figure 6). The HLB-affected trees irrigated at 100% ET showed a 25% greater root volume than those irrigated at 80% ET. Considering root length and area, HLB-affected trees irrigated at 100% ET had at least 20% greater values than those irrigated at 80% ET (Figure 6).

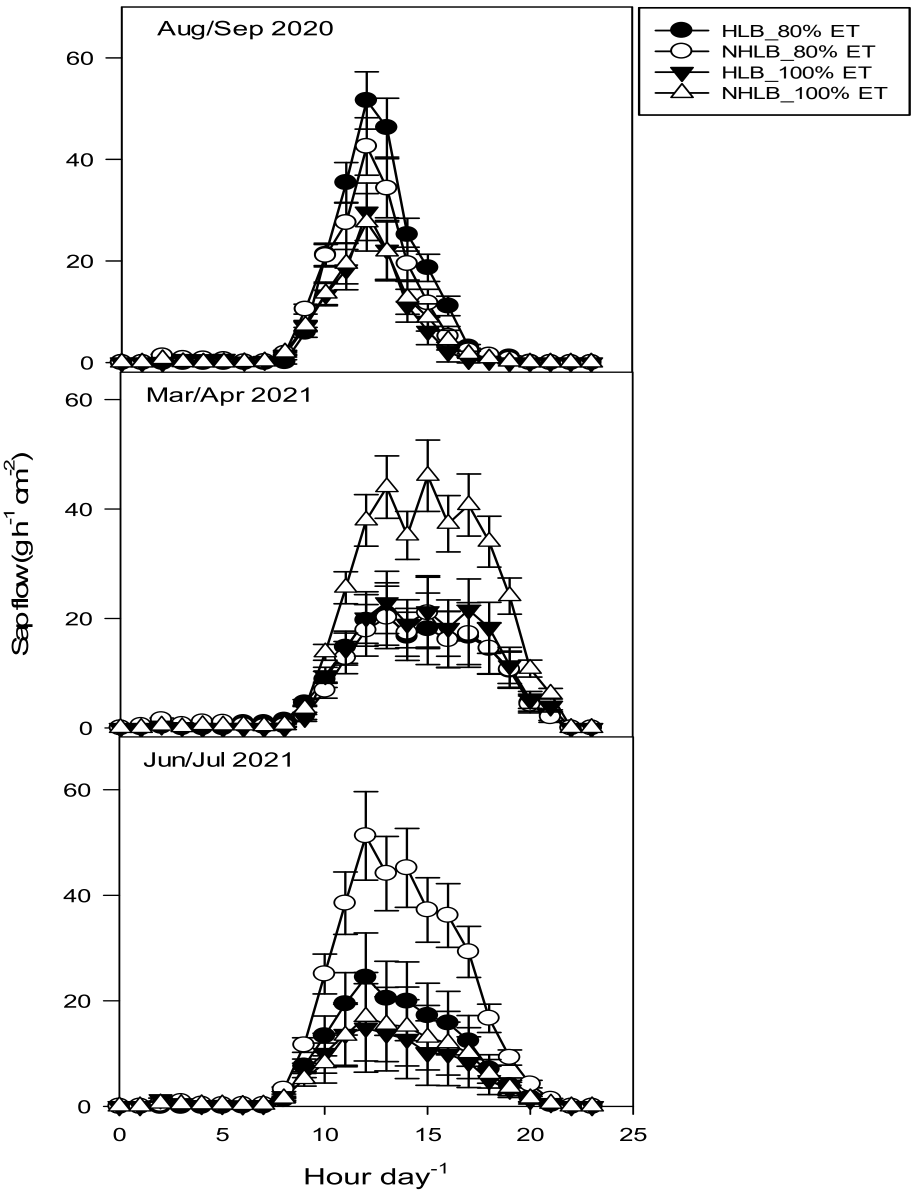

Figure 5. Effect of sap flow (g h^{-1} cm^{-2}) on huanglongbing (HLB)-affected and non-HLB-affected (NHLB) "cv. Valencia" trees under two evapotranspiration (ET)-based irrigation rates (100% and 80% ET) from 2019 to 2020. The data presented are the least squares means for a 24 h period ± SE.

Table 4. Regression of citrus sap flow (g h^{-1} cm^{-2}) per 24 h average on huanglongbing (HLB)-affected and non-HLB-affected (NHLB) "cv. Valencia" orange trees at two evapotranspiration (ET)-based irrigation rates (100% and 80% ET) and three measurement periods using a second-order polynomial model $Y = Y_o + aX + bX^2$.

Treatment	[1] Y_o	[2] A	[2] B	R^2	[3] RMSE (g h^{-1} cm^{-2})	*p*-Value [3]
			August–September 2020			
HLB_100	−5.3	2.7	−0.11	0.37	6.0	**
HLB_80	−11.6	5.2	−0.22	0.37	12.9	**
NHLB_100	−5.8	2.8	−0.12	0.41	6.5	**
NHLB_80	−8.3	4.1	−0.18	0.39	10.0	**
Pooled	−7.7	3.7	−0.16	0.35	9.3	**

Table 4. *Cont.*

Treatment	[1] Y_o	[2] A	[2] B	R^2	[3] RMSE ($g\ h^{-1}\ cm^{-2}$)	*p*-Value [3]
			March–April 2021			
HLB_100	−8.6	3.3	−0.12	0.53	6.5	***
HLB_80	−7.7	3.1	−0.12	0.58	5.3	***
NHLB_100	−16.6	6.4	−0.23	0.52	12.8	***
NHLB_80	−6.9	2.9	−0.11	0.51	5.7	***
Pooled	−10.0	3.9	−0.14	0.42	8.9	***
			June–July 2021			
HLB_100	−4.8	2.3	−0.02	0.59	2.4	***
HLB_80	−8.1	3.5	−0.14	0.56	5.9	***
NHLB_100	−5.6	2.4	−0.10	0.57	4.1	***
NHLB_80	−17.1	7.3	−0.29	0.55	12.7	***
Pooled	−8.9	3.9	−0.15	0.38	9.1	***

Notes: [1] $Y = Y_o + aX + bX^2$, where X is the time of sap flow reading (h), Y_o is the Y intercept [2]. A and B are the regression coefficients for time X. [3] RMSE is the root mean square error. ** and *** stand for *p*-values of 0.01 and 0.001, respectively.

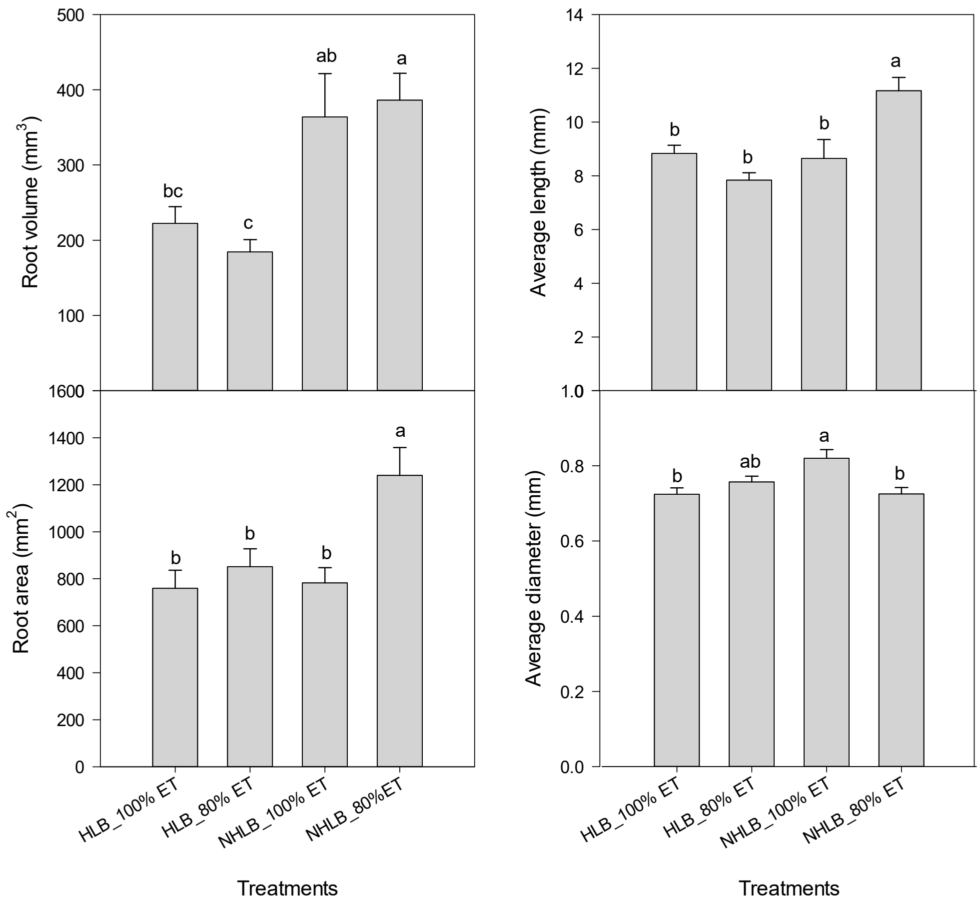

Figure 6. Effect of root growth (area, volume, diameter, and length) on huanglongbing (HLB)-affected and non-HLB-affected (NHLB) "cv. Valencia" orange trees under two evapotranspiration (ET)-based irrigation rates (100% and 80% ET) from 2019 to 2021. Means within a treatment followed by the same letter are not significantly different at $\alpha = 0.05$ (Tukey post hoc tests, $p \leq 0.05$).

4. Discussion

Citrus trees need water for growth and development, and successful irrigation in citrus production could be achieved by maximizing water-use efficiency [9,21,29,30]. HLB has a negative impact on the fibrous root of affected citrus trees; thus, the severity of the disease could be observed not only above ground but also on the root system [8,9]. In this study, a 20% deficit in irrigation was compared to a full irrigation schedule in HLB-affected sweet orange. From our results, healthy trees used about 20% more water than the HLB-affected trees. This could be because of the reduced root volume in HLB-affected trees [9–12]. The findings on growth parameters (height and diameter) showed that all trees responded to growth with time, irrespective of irrigation rate and tree status. However, there was no difference between HLB-affected trees irrigated at 80% ET and 100% ET for both years. The reason for similar growth between the trees subjected to 100% ET and 80% ET could partially be explained by the root growth between the two treatments (Figure 6), similar to what was reported by [17] using 80% ET irrigation. The root volume, root area, average length, and average diameter were similar between the trees subjected to 100% ET and 80% ET. Because the bacteria (*C*Las) start multiplying in the roots [10,13], it may have caused a decline in root biomass that led to a lower root area, hence less water uptake when compared to the healthy (NHLB) trees. For HLB-affected trees, the growth and development of the tree are vital and positively correlated with yield [31]. Therefore, any alteration in cultural practice and irrigation management must be geared towards better growth [21,30]. The canopy development of young HLB-affected trees could be impacted by water stress [21,32]. The author of [32] explained that growth could be slowed in young citrus trees by the slightest water stress experienced. From this study, it was observed that HLB-affected trees had SWP above −1.0 MPa for August and October 2019 and March 2020 as compared with NHLB trees. This may have led to the differences observed in height and diameter between HLB-affected and NHLB trees.

In general, there was no difference observed for SWP between the HLB-affected trees subjected to deficit irrigation and full irrigation, in agreement with results from [33] on Valencia orange, though their trees were not impacted by HLB. This means that the stress level of the HLB-affected trees was not determined by the fact that one set received a 20% deficit in irrigation. The reason for this result could be linked to similar root growth and/or leaf area. As discussed earlier, HLB causes more than a 40% reduction of the fibrous root system of affected trees, which may in turn affect water uptake by the tree [8,9]. This result now provides partial evidence that a 20% deficit in irrigation may not cause HLB-affected trees much more stress than those under full irrigation. Overall, these findings are in accordance with those reported by [13], where less stressed trees had a SWP below −1 MPa and around −2 MPa for trees that showed some stress. Other authors reported SWPs of −3 MPa for seasonal irrigated trees and 1 MPa for pruned trees under irrigation [29,34].

Sap flow measurement for March–April and June–July showed that NHLB trees subjected to 100% ET and 80% ET, respectively, had greater sap flow as compared to HLB-affected trees subjected to both 100% and 80% ET. These results coincide with those observed from the root growth (Figure 6), because the root volume of the NHLB trees tended to be greater than that of HLB-affected trees. This could partially explain why NHLB trees at both irrigation rates showed at least a 28% greater sap flow when compared to HLB-affected trees for the periods of March–April and June–July 2021. The sap flow results further confirmed that the root loss caused by HLB has a negative impact on water uptake. Sap flow, specifically for the periods of March–April and June–July 2021, was comparable between HLB-affected trees at all irrigation rates. The results observed from tree height and trunk diameter could also explain why sap flow was comparable between HLB-affected trees subjected to both full and deficit irrigation. In support of these results, a study by [35] on sap flow and variations in water use in apple orchards showed that canopy cover affects changes in sap flow influx. Some authors reported variations in sap flow influx due to changes in weather conditions [36,37]. However, the variability observed in the results of this study was not mainly due to weather conditions but rather to root growth, changes in tree height, and trunk diameter. The latter may be true only because this study was conducted in a controlled environment.

Maximum sap flow occurred between 11 and 16 h for HLB-affected trees during the three measurement periods (Figure 5). This suggests that water availability at this time has a greater chance of being taken up by the plant. Some authors reported similar sap flow patterns in their studies on citrus irrigation scheduling [13,38]. For example, the work by [38] reported that irrigation events between 9 h and 18 h should result in increased water uptake. This means that an irrigation schedule after 16 h and before 7 h may result in low water uptake for citrus production.

5. Conclusions

Because HLB affects the root system that facilitates water uptake, there was a significant reduction in the root volume and area of HLB-affected citrus trees. The findings of this study revealed that a 20% water reduction for HLB-affected trees may not impact tree growth or increase the stress level as a result of HLB. This study also concluded that the appropriate time to schedule irrigation was between 7 h and 9 h to take advantage of the sap flow influx between 11 h and 16 h. This study showed that for "cv. Valencia" citrus trees affected by HLB, irrigating at 80% ET may be appropriate for achieving an equivalent of 0.5 mm/day of water savings in citrus production under controlled environments. However, since these results were conducted under greenhouse conditions in pots, a follow-up study is needed to validate these results under on-farm field conditions.

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