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## Impact of Deficit Irrigation on Growth and Water-use Dynamics of HLBaffected Citrus Trees

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#### **Research Article**

Keywords: Citrus sinensis, evapotranspiration (ET), Huanglongbing (HLB), sap flow, stem water potential (SWP), water-use efficiency

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### Abstract

A greenhouse study was conducted from October 2019 to July 2021 at the Citrus Research and Education Center (CREC) in Lake Alfred, FL. The objective was to assess the impact of deficit irrigation on tree growth, soil water availability, stem water potential (SWP), sap flow and root growth of 2- to 4-year-old Huanglongbing (HLB)-affected 'Valencia' (*Citrus sinensis*) orange trees on 'Kuharske citrange' rootstock (*Citrus sinensis* x *Poncirus trifoliata*) using evapotranspiration (ET)-based irrigation schedule. A total of 20 trees were either HLB-positive or non-HLB affected and one-half of the trees was subjected to deficit irrigation (80% ET) and the other half to full irrigation (100% ET). For HLB-affected trees irrigated at 80% ET and 100% ET, there was no significant difference for tree height in both years. In general, there was no difference for SWP between the HLB-affected trees subjected to deficit irrigation. Non-HLB trees subjected to 100% ET and 80% ET had greater sap flow compared to HLB-affected trees at all irrigation rates. Maximum sap flow occurred between 11 and 16 h for HLB-affected trees during for the three measurement periods. HLB-affected trees used an average of 1.6 mm day<sup>-1</sup> while non-HLB trees used 2.1 mm day<sup>-1</sup>. On average, healthy trees (non-HLB) used about 20% more water than HLB-affected trees. For HLB-affected trees, irrigating at 80% ET may be appropriate for achieving water savings under controlled environments.

#### Introduction

In most parts of the world, alternative methods are under evaluation to improve water-use efficiency as water becomes scarce <sup>1</sup>. Water-use efficiency in this context is the plant productivity to the amount of water used. Optimal irrigation scheduling, including use of deficit irrigation management, offers an opportunity to reduce water and energy cost, thus potentially increasing water-use and profit margin <sup>2,3</sup>. For citrus production, this means that less water is used to either achieve the same or improved 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* (*C*Las), which is transmitted by an insect vector called Asian citrus psyllid (*Diaphorina citri*) <sup>4–6</sup>.

In Florida, HLB is responsible for over 70% decline in citrus production from 2006 to 2018<sup>7</sup>. The roots of the citrus tree are the first site of bacteria multiplication <sup>8</sup>, and eventually leading to more than 40% loss of fibrous roots (Ebel et al., 2019; Graham et al., 2013; Kadyampakeni et al., 2014; Kadyampakeni et al., 2014). Due to the root loss, an infected tree may not require similar amounts of water like a healthy tree, hence, influencing the tree water-use efficiency (Ebel et al., 2019; Hamido et al., 2017). In Florida, there has been very few research efforts that investigated into HLB-affected trees and water-use dynamics <sup>9,13</sup>. 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 deficit irrigation schedule, because accurate estimation of water-use and stress dynamics could improve irrigation management in citrus production (Kadyampakeni et al., 2014a; Kadyampakeni et al., 2014b; Morgan et al., 2006).

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, and 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 <sup>15,16</sup>. 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 high degree of accuracy. This is important because irrigation scheduling must be both technically and economically efficient and feasible <sup>2</sup>. An irrigation system is deemed economical if it either increases yield or reduces operational costs <sup>3</sup>. 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 'Valencia' (*Citrus sinensis*) sweet orange trees on 'Kuharske citrange' rootstock (*Citrus sinensis x Poncirus trifoliata*) using evapotranspiration-based irrigation in Florida. We hypothesized that HLB-affected citrus trees require less amount of irrigation water to complete their biological functions than healthy citrus trees because of severe fibrous root loss.

# Materials And Methods

## 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 Valencia (*Citrus sinensis*) trees on Kuharske citrange (*Citrus sinensis x Poncirus trifoliata*) root stock. A total of twenty trees including 10 HLB-affected and 10 healthy trees (hereafter called NHLB) were used. About 76 L-size pots were filled with a potting mix and each tree was transplanted into a pot. The potting mix had compost, perlite, bark, and vermiculite. A potting mix was used because of the complexity (drainage system) of managing large volume of sandy soil in lysimeters, in a controlled environment.

Fertilizer was applied in three splits per year at 135 kg N ha<sup>-1</sup> of calcium nitrate and diammonium phosphate, 67 kg P ha<sup>-1</sup> of diammonium phosphate and 100 kg ha<sup>-1</sup> of potassium sulfate. Other essential nutrients were applied following recommendations by Morgan and Kadyampakeni (2020). Thus, fertilization was done in August and December of 2019; April, August and December in 2020; and April in 2021.

### Irrigation water requirement

Each pot was connected to a drip irrigation system with an emitter rate of 4.5 liters 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 were collected from the Florida Automated Weather Network (FAWN) station that was located 350 m from the greenhouse. Daily reference evapotranspiration ( $ET_o$ ) was calculated from FAWN using Penman-Monteith56 method as described by Zotarelli et al. (2010) using Eq. 1. The calculated  $ET_o$  was then multiplied average K<sub>c</sub> values for healthy trees according to Hamido et al (2017) to estimate daily crop evapotranspiration ( $ET_c$ ) according to Eq. 2 (Table 1). Each pot was covered with much and irrigation events occurred between 7 h and 8 h to minimize surface evaporation.

$$ETo = \frac{0.40\Delta (\text{Rn}-\text{G}) + \Upsilon \frac{900}{\text{T}+273} \text{U2 (es-ea)}}{\Delta + \Upsilon (1 + 0.3 \text{u2})}$$
(1)

where  $\text{ET}_{o}$  = reference evapotranspiration, (mm.d<sup>-1</sup>),  $\text{R}_{n}$  = net radiation at the crop surface (MJ.m<sup>-2</sup>.d<sup>-1</sup>), G = soil heat flux density (MJ.m<sup>-2</sup>.d<sup>-1</sup>), T = mean daily air temperature at 2 m height (°C),  $u_{2}$  = wind speed at 2 m height (m.s<sup>-1</sup>),  $e_{s}$  = 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<sup>-1</sup>),  $\gamma$  = psychometric constant (kPa.°C<sup>-1</sup>).

$$ETc = Kc imes ETc$$

2

Where  $ET_c = crop evapotranspiration$ , (mm.d<sup>-1</sup>)  $K_c = crop coefficient$ , and  $ET_o = reference evapotranspiration$  (mm.d<sup>-1</sup>).

Table 1
Estimated monthly citrus water use (ETc) requirement from a 10-year average reference evapotranspiration (ET <sub>o</sub> ). Parameters for ET <sub>o</sub> estimation
retrieved from Florida Automated Weather Network (FAWN) station.

Month	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Avg ET <sub>o</sub>	K <sub>c</sub>	ET <sub>c</sub>	IWR
	ET <sub>o</sub> mm/month ET <sub>o</sub> 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
Avg ET <sub>o</sub> is the average reference evapotranspiration from January 2009 to September 2019.															
K <sub>c</sub> is the citrus coefficient as described by Hamido et al (2017).															
ET <sub>c</sub> is the crop water requirement for equivalent to 100% ET.															
IWR is the estimated irrigation water requirement (IWR) assuming 90% irrigation efficiency. For pots subjected to 80% ET, an 80% of the IWR estimated for the 100% ET was calculated.															

### **Experimental Design**

The experiment was conducted in a randomized complete block design. Two irrigation treatments equivalent to a 100% evapotranspiration (ET) and 80% ET to HLB-affected and non HLB-affected (NHLB) trees were applied. Each ET x tree status (HLB-affected or NHLB) combination was replicated

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

Treatment	Irrigation requirement by crop evapotranspiration (ET, %)	Tree status
1	100	HLB
2	100	NHLB
3	80	HLB
4	80	NHLB

### **Meteorological Measurements**

An automatic weather station (Davis Pro2, Hayward, CA) was mounted in the greenhouse at a 2 m height to measure weather parameters following procedures by Allen et al (1998). The average solar radiation, minimum and maximum air temperature, mean air temperature and relative humidity (RH) were calculated from the weather station data. Daily reference evapotranspiration ( $ET_o$ ) was calculated for the greenhouse using Hargreaves method as described in Eq. 3<sup>1</sup>.

$$ETo = 0.023 \left( 0.408 
ight) \left( Tmean + 17.8 
ight) \left( Tmax - Tmin 
ight)^{0.5} Ra$$

3

where  $T_{max}$  = maximum air temperature (°C),  $T_{min}$  = minimum air temperature (°C),  $R_a$  = solar radiation (MJ m<sup>-2</sup>), and 0.408 is a factor to convert MJ m<sup>-2</sup> to mm.

### **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) 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 before treatment application measurement from subsequent measurements.

### Leaf Area Measurement

Initial and final leaf areas were measured using ImageJ, a Java-based image processing program as described by Schneider et al (2012). Twenty fully expanded leaves were randomly selected from each tree and scanned with an HP scanner (HP ScanJet Pro 2500 f1, Palo, CA) and saved as JPEG images. At the time of leaf sampling, a total leaf count for each tree was also done. The saved JPEG images were then imported into the ImageJ application (https://imagej.nih.gov/ij/download.html) 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 total leaf area.

### Soil Water Content

Soil water content was measured every 30 minutes for the duration of the experiment by two-pronged capacitance sensors (EC-5, Metergroup, Pulman, WA) connected to EM-50 data logger (Meter group, Pulman, WA). 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 replicates for each treatment. Average soil moisture content was calculated from the 3-sensors on each treatment.

## Stem Water Potential (SWP)

The stem water potential was measured using a portable pressure chamber (Model 1505D, PMS Instrument Company-Albany, OR). The measurements were done using a similar procedure described by Fulton (2014) for higher plants. Four representative leaves per tree (two trees per treatment) were randomly selected and covered with an 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 recorded (MPa).

### Water Use Dynamics

Water use was determined using sap flow measurements taken on 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). Stem diameters for the measurements ranged from about 10.1 mm to 16.6 mm during the study. A silicon grease was used to improve 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<sup>-2</sup>. A 24 h daily water flow was calculated for each measuring period and compared among treatments.

## **Root Growth**

Root growth was assessed monthly using transparent acrylic minirhizotrons installed in each plot using methods described by Han et al. (2016). The minirhizotrons were installed either to the east or west of the trunk, along the direction of the drip emitter at  $45^{\circ}$  angle at 20 cm away from the tree's trunk to a depth of 50 cm from the surface. The CID-600 root imager (CID-Bioscience, Pullman, WA) was then used to scan roots within the visible area (21 × 19 = 399 cm<sup>2</sup>) of the minirhizotron to estimate root diameter, length, area, and volume and the results were compared among treatments.

# Data Analysis

The two irrigation rates (ET = 100% and ET = 80%) were considered as blocks and tree status (HLB-affected and NHLB) as subplots. Analysis of variance (ANOVA), using the generalized linear mixed model procedure (PROC GLIMMIX) as implemented in SAS (SAS/STAT 15.1, SAS Institute, Cary, NC [2018]) was used to analyze all response data. When significant (at  $\alpha = 0.05$ ), a multiple comparison by Tukey's post hoc honest significance difference test was performed. Correlations and linear regression between variables were determined using Sigma Plot software (version 12.3; Systat Software Inc, San Jose, CA). An unstructured covariance model (UN) was chosen as a best fit to model the repeated nature of some parameters based on Akaike's Information Criterion corrected for small sample size (AICC). Response variable measured at the end of the experiment was analyze based on a complete factorial combination of treatment factors irrigation rate and tree status. Visual inspection of residuals <sup>17</sup> indicated no violations of the underlying assumptions.

### Results

### **Meteorological Measurements**

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<sup>-2</sup> day<sup>-1</sup> from 2019 to 2021. The calculated  $ET_o$  from the greenhouse weather station was between 1.22 mm day<sup>-1</sup> for the days with minimum air temperatures and 3.99 mm day<sup>-1</sup> for days with maximum air temperature. 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 day<sup>-1</sup> were calculated for healthy trees, with an average of 2.1 mm day<sup>-1</sup>. Similarly, ETc values ranging from 0.7 to 3.0 mm day<sup>-1</sup> was calculated for HLB-affected trees with an average of 1.6 mm day<sup>-1</sup>.

Oct_19       27.3         Nov_19       22.9         Dec_19       22.0         Jan_20       21.7         Feb_20       22.3         Mar_20       27.9         Jun_20       28.8         Jul_20       29.4         Aug_20       29.4         Sep_20       30.2	•C 3 ± 1.2 9 ± 1.9 0 ± 2.6 7 ± 2.7 9 ± 1.9 7 ± 2.1 9 ± 1.7 8 ± 2.2 4 ± 2.7	$ \begin{array}{c}$	$26.7 \pm 1.2$ $22.4 \pm 1.9$ $21.5 \pm 2.5$ $21.2 \pm 2.7$ $22.3 \pm 1.9$ $22.2 \pm 2.0$ $27.3 \pm 1.6$ $28.2 \pm 2.2$ $28.8 \pm 2.7$	$(\%)$ $73.9 \pm 4.3$ $68.9 \pm 0.9$ $71.7 \pm 2.8$ $64.2 \pm 2.5$ $62.9 \pm 3.4$ $56.7 \pm 7.8$ $69.1 \pm 1.0$ $69.4 \pm 1.2$	(MJ m-2 day-1)5.0 ± 0.44.5 ± 0.83.4 ± 1.64.1 ± 1.15.1 ± 0.46.4 ± 0.66.9 ± 0.97.1 ± 1.0	$\begin{array}{c} (r \\ 2.2 \pm \\ 0.2 \\ 1.8 \pm \\ 0.5 \\ 1.2 \pm \\ 0.9 \\ 1.5 \pm \\ 0.7 \\ 2.0 \pm \\ 0.4 \\ 2.4 \pm \\ 0.0 \\ 3.2 \pm \\ 0.5 \\ 3.3 \pm \\ 0.6 \\ \end{array}$	mm day <sup>-1</sup> ) 2.1 $\pm$ 0.1 1.5 $\pm$ 0.4 1.0 $\pm$ 0.8 1.4 $\pm$ 0.5 1.8 $\pm$ 0.3 2.0 $\pm$ 0.1 2.4 $\pm$ 0.2 2.7 $\pm$ 0.4	1.6 ± 0.0 ± 1.1 ± 0.3 ± 0.7 ± 0.6 ± 1.1 ± 0.4 ± 1.5 ± 0.1 ± 0.4 ± 0.1 ± 0.4 ± 0.1 ± 0.4 ± 0.1 ± 0.4 ± 0.1 ± 0.4 ± 0.1 ± 0.4 ± 0.1 ± 0.4 ± 0.1 ± 0.1 ± 0.4 ± 0.1 ± 0.4 ± 0.1 ± 0.1 ± 0.4 ± 0.1 ±
Oct_19       27.3         Nov_19       22.9         Dec_19       22.0         Jan_20       21.7         Feb_20       22.9         Mar_20       27.9         Jun_20       28.8         Jul_20       29.4         Aug_20       29.4         Sep_20       30.2	$3 \pm 1.2$ $9 \pm 1.9$ $0 \pm 2.6$ $7 \pm 2.7$ $9 \pm 1.9$ $7 \pm 2.1$ $9 \pm 1.7$ $8 \pm 2.2$ $4 \pm 2.7$	$26.2 \pm 1.2$ $21.9 \pm 1.8$ $21.0 \pm 2.4$ $20.7 \pm 2.7$ $21.8 \pm 1.9$ $21.7 \pm 2.0$ $26.7 \pm 1.6$ $27.6 \pm 2.2$ $28.2 \pm 2.7$	$26.7 \pm 1.2$ $22.4 \pm 1.9$ $21.5 \pm 2.5$ $21.2 \pm 2.7$ $22.3 \pm 1.9$ $22.2 \pm 2.0$ $27.3 \pm 1.6$ $28.2 \pm 2.2$ $28.8 \pm 2.7$	$73.9 \pm  4.3  68.9 \pm  0.9  71.7 \pm  2.8  64.2 \pm  2.5  62.9 \pm  3.4  56.7 \pm  7.8  69.1 \pm  1.0  69.4 \pm  1.2 $	$5.0 \pm 0.4$ $4.5 \pm 0.8$ $3.4 \pm 1.6$ $4.1 \pm 1.1$ $5.1 \pm 0.4$ $6.4 \pm 0.6$ $6.9 \pm 0.9$ $7.1 \pm 1.0$	$\begin{array}{c} 2.2 \pm \\ 0.2 \\ \hline 0.3 \\ \hline 0.4 \\ \hline 0.5 \\ \hline 0.5 \\ \hline 0.5 \\ \hline 0.3 \\ \hline 0.6 \\ \hline \end{array}$	2.1 $\pm$ 0.1 1.5 $\pm$ 0.4 1.0 $\pm$ 0.8 1.4 $\pm$ 0.5 1.8 $\pm$ 0.3 2.0 $\pm$ 0.1 2.4 $\pm$ 0.2 2.7 $\pm$ 0.4	$ \begin{array}{c} 1.6 \pm \\ 0.0 \\ 1.1 \pm \\ 0.7 \pm \\ 0.6 \\ 1.1 \pm \\ 0.4 \\ 1.5 \pm \\ 0.1 \\ 1.6 \pm \\ 0.1 \\ 1.7 \pm \\ 0.1 \\ 1.9 \pm \\ 1.$
Nov_19         22.9           Dec_19         22.0           Jan_20         21.7           Feb_20         22.9           Mar_20         22.7           Jun_20         28.8           Jul_20         29.4           Aug_20         29.7           Sep_20         30.2	$9 \pm 1.9$ $0 \pm 2.6$ $7 \pm 2.7$ $9 \pm 1.9$ $7 \pm 2.1$ $9 \pm 1.7$ $8 \pm 2.2$ $4 \pm 2.7$	21.9 $\pm$ 1.8 21.0 $\pm$ 2.4 20.7 $\pm$ 2.7 21.8 $\pm$ 1.9 21.7 $\pm$ 2.0 26.7 $\pm$ 1.6 27.6 $\pm$ 2.2 28.2 $\pm$ 2.7	$22.4 \pm 1.9$ $21.5 \pm 2.5$ $21.2 \pm 2.7$ $22.3 \pm 1.9$ $22.2 \pm 2.0$ $27.3 \pm 1.6$ $28.2 \pm 2.2$ $28.8 \pm 2.7$	$ \begin{array}{c} 68.9 \pm \\ 0.9 \\ \hline 71.7 \pm \\ 2.8 \\ \hline 64.2 \pm \\ 2.5 \\ \hline 62.9 \pm \\ 3.4 \\ \hline 56.7 \pm \\ 7.8 \\ \hline 69.1 \pm \\ 1.0 \\ \hline 69.4 \pm \\ 1.2 \\ \end{array} $	$\begin{array}{c} 4.5 \pm 0.8 \\ 3.4 \pm 1.6 \\ 4.1 \pm 1.1 \\ 5.1 \pm 0.4 \\ 6.4 \pm 0.6 \\ 6.9 \pm 0.9 \\ 7.1 \pm 1.0 \end{array}$	$ \begin{array}{c} 1.8 \pm \\ 0.5 \\ 1.2 \pm \\ 0.9 \\ 1.5 \pm \\ 0.7 \\ 2.0 \pm \\ 0.4 \\ 2.4 \pm \\ 0.0 \\ 3.2 \pm \\ 0.5 \\ 3.3 \pm \\ 0.6 \\ \end{array} $	$\begin{array}{c} 1.5 \pm 0.4 \\ 1.0 \pm 0.8 \\ 1.4 \pm 0.5 \\ 1.8 \pm 0.3 \\ 2.0 \pm 0.1 \\ 2.4 \pm 0.2 \\ 2.7 \pm 0.4 \end{array}$	$ \begin{array}{c} 1.1 \pm \\ 0.3 \\ 0.7 \pm \\ 0.6 \\ \end{array} $ $ \begin{array}{c} 1.1 \pm \\ 0.4 \\ \end{array} $ $ \begin{array}{c} 1.5 \pm \\ 0.1 \\ \end{array} $ $ \begin{array}{c} 1.6 \pm \\ 0.1 \\ \end{array} $ $ \begin{array}{c} 1.7 \pm \\ 0.1 \\ \end{array} $
Dec_19       22.0         Jan_20       21.7         Feb_20       22.9         Mar_20       27.9         Jun_20       28.8         Jul_20       29.4         Aug_20       29.4         Sep_20       30.2	0 ± 2.6 7 ± 2.7 9 ± 1.9 7 ± 2.1 9 ± 1.7 8 ± 2.2 4 ± 2.7	21.0 $\pm$ 2.4 20.7 $\pm$ 2.7 21.8 $\pm$ 1.9 21.7 $\pm$ 2.0 26.7 $\pm$ 1.6 27.6 $\pm$ 2.2 28.2 $\pm$ 2.7	$21.5 \pm 2.5$ $21.2 \pm 2.7$ $22.3 \pm 1.9$ $22.2 \pm 2.0$ $27.3 \pm 1.6$ $28.2 \pm 2.2$ $28.8 \pm 2.7$	$71.7 \pm 2.8$ $64.2 \pm 2.5$ $62.9 \pm 3.4$ $56.7 \pm 7.8$ $69.1 \pm 1.0$ $69.4 \pm 1.2$	$3.4 \pm 1.6$ $4.1 \pm 1.1$ $5.1 \pm 0.4$ $6.4 \pm 0.6$ $6.9 \pm 0.9$ $7.1 \pm 1.0$	$ \begin{array}{c} 1.2 \pm \\ 0.9 \\ 1.5 \pm \\ 0.7 \\ 2.0 \pm \\ 0.4 \\ 2.4 \pm \\ 0.0 \\ 3.2 \pm \\ 0.5 \\ 3.3 \pm \\ 0.6 \\ \end{array} $	$1.0 \pm 0.8$ $1.4 \pm 0.5$ $1.8 \pm 0.3$ $2.0 \pm 0.1$ $2.4 \pm 0.2$ $2.7 \pm 0.4$	0.7± 0.6 1.1± 0.4 1.5± 0.1 1.6± 0.0 1.7± 0.1
Jan_20       21.7         Feb_20       22.9         Mar_20       22.7         May_20       27.9         Jun_20       28.8         Jul_20       29.4         Aug_20       29.7         Sep_20       30.2	7 ± 2.7 9 ± 1.9 7 ± 2.1 9 ± 1.7 8 ± 2.2 4 ± 2.7	20.7 ± 2.7 21.8 ± 1.9 21.7 ± 2.0 26.7 ± 1.6 27.6 ± 2.2 28.2 ± 2.7	$21.2 \pm 2.7$ $22.3 \pm 1.9$ $22.2 \pm 2.0$ $27.3 \pm 1.6$ $28.2 \pm 2.2$ $28.8 \pm 2.7$	$ \begin{array}{c} 64.2 \pm \\ 2.5 \\ 62.9 \pm \\ 3.4 \\ 56.7 \pm \\ 7.8 \\ 69.1 \pm \\ 1.0 \\ 69.4 \pm \\ 1.2 \\ \end{array} $	$4.1 \pm 1.1$ $5.1 \pm 0.4$ $6.4 \pm 0.6$ $6.9 \pm 0.9$ $7.1 \pm 1.0$	$ \begin{array}{c} 1.5 \pm \\ 0.7 \\ 2.0 \pm \\ 0.4 \\ \end{array} $ $ \begin{array}{c} 2.4 \pm \\ 0.0 \\ 3.2 \pm \\ 0.5 \\ 3.3 \pm \\ 0.6 \\ \end{array} $	$\begin{array}{c} 1.4 \pm 0.5 \\ 1.8 \pm 0.3 \\ 2.0 \pm 0.1 \\ 2.4 \pm 0.2 \\ 2.7 \pm 0.4 \end{array}$	1.1± 0.4 1.5± 0.1 1.6± 0.0 1.7± 0.1
Feb_20       22.5         Mar_20       22.7         May_20       27.9         Jun_20       28.8         Jul_20       29.4         Aug_20       29.7         Sep_20       30.2	9±1.9 7±2.1 9±1.7 8±2.2 4±2.7	21.8 ± 1.9 21.7 ± 2.0 26.7 ± 1.6 27.6 ± 2.2 28.2 ± 2.7	$22.3 \pm 1.9$ $22.2 \pm 2.0$ $27.3 \pm 1.6$ $28.2 \pm 2.2$ $28.8 \pm 2.7$	$ \begin{array}{c} 62.9 \pm \\ 3.4 \\ 56.7 \pm \\ 7.8 \\ 69.1 \pm \\ 1.0 \\ 69.4 \pm \\ 1.2 \\ \end{array} $	$5.1 \pm 0.4$ $6.4 \pm 0.6$ $6.9 \pm 0.9$ $7.1 \pm 1.0$	$2.0 \pm \\ 0.4$ $2.4 \pm \\ 0.0$ $3.2 \pm \\ 0.5$ $3.3 \pm \\ 0.6$	$1.8 \pm 0.3$ 2.0 ± 0.1 2.4 ± 0.2 2.7 ± 0.4	1.5± 0.1 1.6± 0.0 1.7± 0.1
Mar_20       22.7         May_20       27.9         Jun_20       28.8         Jul_20       29.4         Aug_20       29.7         Sep_20       30.2	7 ± 2.1 9 ± 1.7 8 ± 2.2 4 ± 2.7	21.7 ± 2.0 26.7 ± 1.6 27.6 ± 2.2 28.2 ± 2.7	$22.2 \pm 2.0$ $27.3 \pm 1.6$ $28.2 \pm 2.2$ $28.8 \pm 2.7$	56.7 ±           69.1 ±           1.0           69.4 ±           1.2	$6.4 \pm 0.6$ $6.9 \pm 0.9$ $7.1 \pm 1.0$	$2.4 \pm 0.0$ 3.2 \pm 0.5 3.3 \pm 0.6	$2.0 \pm 0.1$ $2.4 \pm 0.2$ $2.7 \pm 0.4$	1.6± 0.0 1.7± 0.1
May_20 27.9 Jun_20 28.8 Jul_20 29.4 Aug_20 29.7 Sep_20 30.2	9 ± 1.7 8 ± 2.2 4 ± 2.7	26.7 ± 1.6 27.6 ± 2.2 28.2 ± 2.7	27.3 ± 1.6 28.2 ± 2.2 28.8 ± 2.7	69.1 ± 1.0 69.4 ± 1.2	6.9 ± 0.9 7.1 ± 1.0	3.2 ± 0.5 3.3 ± 0.6	2.4 ± 0.2 2.7 ± 0.4	1.7± 0.1
Jun_20     28.8       Jul_20     29.4       Aug_20     29.7       Sep_20     30.2	8 ± 2.2 4 ± 2.7	27.6 ± 2.2 28.2 ± 2.7	28.2 ± 2.2 28.8 ± 2.7	69.4± 1.2	7.1 ± 1.0	3.3 ± 0.6	2.7 ± 0.4	10+
Jul_20 29.4 Aug_20 29.7 Sep_20 30.2	4 ± 2.7	28.2 ± 2.7	28.8 ± 2.7					0.2
Aug_20 29.7 Sep_20 30.2				71.3 ± 2.5	6.7 ± 0.7	3.2 ± 0.5	3.0±0.6	2.0 ± 0.3
Sep_20 30.2	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
	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_21 19.6	6 ± 4.3	18.3 ± 4.3	18.9 ± 4.3	67.5± 0.1	3.7 ± 1.4	1.5 ± 0.7	$1.4 \pm 0.5$	1.1 ± 0.4
Feb_21 22.5	5 ± 2.2	21.3 ± 2.2	21.9 ± 2.2	68.0± 0.2	3.9 ± 1.2	1.6 ± 0.6	$1.4 \pm 0.5$	1.2 ± 0.3
Mar_21 23.6	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_21 25.0	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_21 27.7	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_21 28.8	8 ± 2.2	27.6 ± 2.3	28.2 ± 2.2	69.0± 0.9	6.0 ± 0.2	2.7 ± 0.2	$2.2 \pm 0.0$	1.6± 0.0
Jul_21 28.4	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
ET <sub>o</sub> is reference e	vapotranspiration							

## Tree Height, Trunk Diameter and Leaf Area

Tree height was significantly different (P = 0.009) for both years between treatment. In year one, HLB-affected trees subjected to 100% ET had 5–8% greater height than NHLB trees at 100% and 80% ET, respectively (Fig. 1). However, between the HLB-affected trees, there was no significant difference between tree height for 100% or 80% ET. In the second year, NHLB trees subjected to 80% ET had 5–7% increase in height as compared to HLB-affected trees that were irrigated at 80% and 100% ET (Fig. 1). Results on height between the HLB-affected trees were comparable. HLB-affected trees at both 100% ET and 80% ET had at least 5% increase in trunk diameter than NHLB-affected trees at 100% ET and 80% ET (Fig. 1), at

the end of year one. In the second year, the changes observed in trunk diameter was comparable for all trees irrespective of their HLB status or irrigation rate. Leaf area at the end of the second year was not significantly different (*P* > 0.05) among all treatments (Fig. 2).

## Soil Water Content

Soil water content at the irrigated zone was between 0.10 and 0.35 cm<sup>3</sup> cm<sup>-3</sup> in all the pots. The trees under 100% ET had the highest values, about 30% more moisture content than the trees under 80% ET (Fig. 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.

## **Stem Water Potential**

Stem water potential (SWP) was significantly different (P<0.0001) among treatments and between measurements (P<0.001). The results for the SWP ranged between – 2.4 and – 0.6 MPa. The highest 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 but August 2019 (Fig. 4). However, the NHLB trees under 80% and 100% ET showed significant differences for most of the periods, for example in May 2020, September 2020, November 2020, and July 2021 (Fig. 4).

## Sap Flow Dynamics

In August-September 2020, trees subjected to deficit irrigation schedule showed 12% greater sap flow as compared to trees subjected full irrigation schedule (Fig. 5). However, in March-April 2021, NHLB trees with 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 30% greater sap flow than all other treatments (Fig. 5). Generally, sap flow occurred between 8 and 20 h daily. Sap flow (g  $h^{-1}$  cm<sup>-2</sup>) peaked between 12 h, 12 and 15 h, and 12 h per day in August-September 2020, March-April 2021 and June-July 2021, respectively (Fig. 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 40% greater slope when compared to the trees subjected to full irrigation. Thus, these trees had a greater rise in sap flow per hour increase 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 100% rise in sap flow per hour when compared to other treatments. Trees subjected to 100% ET showed a better fit (with lower RMSE) to the regression model than trees subjected to 80% ET, for the Spring and Summer measurements while in Spring, trees subjected to 80% ET showed a better fit to the regression model.

#### Table 4

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

Treatment	Yo	Α	В	R <sup>2</sup>	RMSE (g $h^{-1}$ cm <sup>-2</sup> )	P-value			
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	**			
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	***			
Y is the intercept, A is coefficient of the first variable, B is coefficient of the second variable,									

R<sup>2</sup> coefficient of determination, RMSE is the root mean square error.

ns, \*, \*\*, \*\*\* represents non-significant, significant at P< 0.05, 0.01, or 0.001, respectively.

### **Root Growth**

Root growth was significantly different among treatments. Total root volume and area ranged between 175 to 491 mm<sup>3</sup> and 795 and 1253 m<sup>2</sup>, respectively (Fig. 6). Root volume for NHLB trees at 80% ET was 50–60% greater than HLB-affected trees at 100% and 80% ET, respectively (Fig. 6). The HLB-affected trees irrigated at 100% ET showed 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 (Fig. 6).

#### Discussion

Citrus trees need water for growth and development, and successful irrigation in citrus production could be achieved by maximizing water-use efficiency <sup>9, 18–20</sup>. From this study, a 20% deficit irrigation was compared to a full irrigation schedule for 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 for HLB-affected trees (Ebel et al., 2019; Graham et al., 2013; Kadyampakeni et al., 2014; Kadyampakeni et al., 2014). 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 differences 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 (Fig. 6). 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 (Graham et al., 2013; Hamido et al., 2017), it may have caused a decline in root biomass that led to fewer root area, hence less water uptake when compared the healthy (NHLB) trees. For HLB-affected trees, the growth and development of the tree is vital and positively correlated to yield <sup>21</sup>. Therefore, any alteration in cultural practice, thus, irrigation management must be geared towards better growth <sup>19,20</sup>. Canopy development of young HLB-affected trees could be impacted by water stress <sup>19,22</sup>. Levy (1998) explained that growth could be slowed in young citrus trees upon 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. This means that the stress level of the HLB-affected trees was not determined by the fact that one set received a 20% deficit irrigation. The reason for this result could be linked to similar root growth and/or the leaf area. This result now provides partial evidence that a 20% deficit irrigation may not cause HLB-affected trees much stress than those under full irrigation. Overall, these findings are in accordance with findings reported by Hamido et al (2017), where less stress trees had SWP below – 1 MPa and around – 2 MPa for trees that showed some stress. Other authors reported SWP of -3 MPa for seasonal irrigated trees and 1 MPa for pruned trees under irrigation <sup>18,23</sup>.

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 results observed from the root growth (Fig. 6) because root volume for the NHLB trees tended to be greater than that of HLB-affected trees. This could partially explain why NHLB trees at both irrigation rate showed at least 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 because of HLB has a negative impact on water uptake. Sap flow, specifically, for the period of March-April and June-July 2021 was comparable between HLB-affected trees at all irrigation rates. The results observed from the 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 this results, a study by Mobe et al. (2020) on sap flow and variations in water use of apple orchards showed that canopy cover affects changes in sap flow influx. Some authors reported variation in sap flow influx due to changes in weather conditions <sup>24,25</sup>. However, the variability observed from results of this study was not mainly due to weather conditions but rather root growth, changes in tree height and trunk diameter. The latter may be true only because this study was conducted under a controlled environment.

Maximum sap flow occurred between 11 and 16 h for HLB-affected trees during the three measurement periods (Fig. 5). This suggests that water availability for this time has a greater chance to be taken up by the plant. Some authors reported similar sap flow patterns on their studies on citrus irrigation scheduling (Hamido et al., 2017; Kadyampakeni & Morgan, 2017). For example, Kadyampakeni and Morgan (2017) reported that irrigation event between 9 h and 18 h should result in increased water uptake. This means that irrigation schedule after 16 h and before 7 h may result in low water uptake for citrus production.

#### Conclusions

As climate change affects water used for agricultural purposes, improved irrigation management may be useful to minimize water waste and reduce operational cost. The findings of this study revealed that the appropriate time to schedule irrigation was between 7 h and 9 h, to take advantage of the sap flow influx between 11 and 16 h. It is well understood that for field conditions, climate parameters for example, high temperatures, radiation and low humidity might affect the average evapotranspiration and sap flow influx. Therefore, when scheduling irrigation in a particular region, climatic factors should be well monitored and considered. This study showed that for 'Valencia' citrus trees affected by HLB, irrigating at 80% ET may be appropriate for achieving 20% water savings in citrus production under controlled environments.

#### **List Of Abbreviations**

ANOVA Analysis of variance

- CREC Citrus Research and Education Center
- ET evapotranspiration
- ET<sub>o</sub> reference evapotranspiration
- K<sub>c</sub> crop coefficient

IFAS Institute of Food and Agricultural Sciences

NASS National Agriculture Statistics Service

RMSE root mean square error

SWP stem water potential

#### Declarations

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### AUTHOR CONTRIBUTIONS

Samuel Kwakye: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. Davie Kadyampakeni: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Visualization, Writing – review & editing.

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#### Figure 1

Percent change in tree height and trunk diameter on huanglongbing (HLB)-affected and non HLB-affected (NHLB) 'Valencia' trees at two evapotranspiration (ET)-based irrigation rates (100% and 80% ET) from 2019 to 2021.



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



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



Effect of stem water potential (MPa) on huanglongbing(HLB)-affected and non HLB (NHLB) 'Valencia' trees under two evapotranspiration (ET)based irrigation (100% and 80% ET) from 2019 to 2020. 1MPa = 10 bar. Means within a treatment followed by the same letter are not significantly different at  $\alpha$  = 0.05 (Tukey post hoc tests, p≤0.05).



Effect of sap flow (g  $h^{-1}$  cm<sup>-2</sup>) on huanglongbing (HLB)-affected and non HLB (NHLB) 'Valencia' trees under two evapotranspiration (ET)-based irrigation rates (100% and 80% ET) from 2019 to 2020. Data presented are the least square means for a 24 h period ± SE.



Effect of root growth (area, volume, diameter, and length) on huanglongbing (HLB)-affected and non HLB-affected (NHLB) '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,  $p \le 0.05$ ).