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Protected Fresh Grapefruit Cultivation Systems: Antipsyllid Screen Effects on Plant Growth and Leaf Transpiration, Vapor Pressure Deficit, and Nutrition

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SUMMARY. Completely enclosed screen houses can physically exclude contact between the asian citrus psyllid [ACP (*Diaphorina citri*)] and young, healthy citrus (*Citrus* sp.) trees and prevent huanglongbing (HLB) disease development. The current study investigated the use of antipsyllid screen houses on plant growth and physiological parameters of young 'Ray Ruby' grapefruit (*Citrus × paradisi*) trees. We tested two coverings [enclosed screen house and open-air (control)] and two planting systems (in-ground and container-grown), with four replications arranged in a split-plot experimental design. Trees grown inside screen houses developed larger canopy surface area, canopy surface area water use efficiency (CWUE), leaf area index (LAI) and LAI water use efficiency (LAIWUE) relative to trees grown in open-air plots ($P < 0.01$). Leaf water transpiration increased and leaf vapor pressure deficit (VPD) decreased in trees grown inside screen houses compared with trees grown in the open-air plots. CWUE was negatively related to leaf VPD ($P < 0.01$). Monthly leaf nitrogen concentration was consistently greater in container-grown trees in the open-air compared with trees grown in-ground and inside the screen houses. However, trees grown in-ground and inside the screen houses did not experience any severe leaf N deficiencies and were the largest trees, presenting the highest canopy surface area and LAI at the end of the study. The screen houses described here provided a better growing environment for in-ground grapefruit because the protective structures accelerated young tree growth compared with open-air plantings while protecting trees from HLB infection.

Citrus producers are fighting HLB, a disease associated with the bacterium *Candidatus Liberibacter asiaticus* [CLas (Bové, 2006)]. The disease was detected in Florida in 2004, and since that time, citrus commercial acreage has decreased from 748,555 to 480,121 acres, and the total citrus production reduced from 291,800,000 to 94,205,000 85-lb boxes in 2015–16

season [U.S. Department of Agriculture (USDA, 2017)]. The 36% reduction of planted area and 68% drop in yield is causing major economic and social problems to the state of Florida. HLB disease affects 90% of Florida's total citrus acreage and on average 80% trees in an individual citrus operation are infected with the pathogen, resulting in 41% yield loss (Singerman

and Useche, 2016). Trees affected by HLB suffer from general canopy and root decline, yield reduction, and lopsided fruit that are not fit for sale on the fresh market. One characteristic of the disease is that yield losses precede visible foliar symptoms (Bassanezi et al., 2011), potentially indicating that fruit production is negatively affected before the disease is visually detected. Because the CLas bacterium is vectored by the ACP, insecticide applications aimed at reducing ACP populations and feeding activity typically constitute the main bulwark of HLB mitigation programs (Bassanezi et al., 2013; Hall et al., 2013; Stansly et al., 2014). The success of an insecticide ACP-management program is affected by the treated area, with greater efficacy usually achieved over larger swaths of land (Bassanezi et al., 2013). Coordinating insecticide applications over large acreages and among different farms adds another layer of complexity to an inefficient HLB-ACP control strategy.

Completely enclosed screen houses physically exclude the ACP and thus prevent inoculation and disease development. One of the main advantages of this system includes decrease in frequency of insecticide sprays to control psyllids. Ferrarezi et al. (2017) found no eggs, nymphs and adult ACP and no trees tested positive for CLas inside protective screen houses after 2 years of monitoring, where 75% of surveyed trees in the open-air plots tested positive for CLas during the same period. Thus, the use of screen houses offered a substantial level of protection against the establishment of HLB within a young grapefruit planting compared with management programs founded solely on insecticidal sprays. The system was developed at the University of Florida/Institute of Food and Agricultural Sciences (UF/IFAS) Indian River Research and Education Center in Fort Pierce, FL, and has been tested at the UF/IFAS Citrus Research and Education Center in Lake Alfred, FL. The economics of citrus under protective screens (CUPS) is being determined (Schumann and Singerman, 2016). To date, there are 50 acres of commercial CUPS with three growers in Florida and at least 150 acres more are planned (E.I. Pines and S.B. Callaham, personal communication).

If protected antipsyllid screen houses are to be used as a strategy by fresh citrus fruit producers to grow HLB-free trees, it must be demonstrated that young tree growth is not impeded by this potential cultivation method. Ferrarezi et al. (2017) reported that monthly rainfall was unaffected by the screen houses compared with open-air plots. Cumulative solar radiation and reference evapotranspiration (ET_o) were reduced within the screen houses by 23% and 21%, respectively, compared with the open-air plots over 5 months of data collection. In Florida citrus production regions, rainfall amounts are typically the greatest from June to September (Parsons and Wheaton, 2000; USDA, 2014), with the rest of the year receiving little to no precipitation. Thus, the meteorological conditions inside the screen houses (no loss of rainfall, decreased solar radiation, and cumulative ET_o) could offer a horticultural benefit to growing young citrus trees undercover by potentially increasing water-use efficiency (WUE).

Containerized production of young citrus trees could offer a novel approach to growing trees within a protected environment for fresh fruit product. A chief advantage of potted production, compared with conventionally growing trees in-ground, is its high degree of possible compartmentalization. For example, if a breach occurred and ACP detected, sprays would commence and the affected container-grown trees could be swiftly removed. Because CLas

has a long incubation period before detection, the impact from a breach may not be detected for several years—which is a serious issue for the production system. Our hypothesis is antipsyllid screen houses and container-grown cultivation would allow rapid young plant growth, playing a role in developing new citrus production systems aiming a vector-free environment. One should note that the screen house itself cannot prevent disease development.

The current study investigated the use of antipsyllid screen houses on plant growth and physiological parameters of young ‘Ray Ruby’ grapefruit trees.

Materials and methods

SITE LOCATION. The study was established in Nov. 2013 at the UF/IFAS Indian River Research and Education Center in Fort Pierce, FL (lat. 27°26′N, long. 80°26′W, elevation 19 ft). Soil at the site is classified in the Pineda series: loamy, siliceous, active hyperthermic Arenic Glossaqualfs.

ANTIPSYLLID SCREEN HOUSES. Four passively ventilated 1/4-acre (100 ft wide × 120 ft long × 14 ft tall) completely enclosed screen houses were constructed using a 50-mesh monofilament high-density polyethylene screen (Signature Supply, Lakeland, FL) (Fig. 1). The main support for each enclosed, covered structure consisted of pressure-treated, wooden utility poles (Outdoor Living Products, Orlando, FL). Each main support utility pole was fixed to the ground with one guy-wire (1/4-inch-diameter, braided, galvanized steel wire) and attached to two 5-ft-long earth anchors (both Pierson Supply, Pierson, FL). Support utility poles located in the corner position of

each screen house were fixed to the ground with two guy-wires and four earth anchors.

The antipsyllid screen was attached to the sides of the structure by stapling the cloth to the interior side of the perimeter main support utility poles. The side screen cloth was attached to the top screen cloth with “S”-shaped galvanized steel hooks, and the side screen panels and top screen panels were pleated together, with the resulting seam directed toward the interior of the house. The construction of each screen house included one aluminum roll-up garage-style service door (8 ft wide × 10 ft tall). A 12-ft-wide × 12-ft-long × 12-ft-tall antechamber was built to limit insect inclusion when the entrance door is opened.

All four antipsyllid screen houses and open-air plots were surrounded by at least 50-ft buffer area to prevent any influence on micrometeorological conditions to the next-nearest screen house and open-air plots.

TREATMENTS AND EXPERIMENTAL DESIGN. We tested two coverings [enclosed screen house and open-air (control)] and two planting systems (in-ground and container-grown), with four replications arranged in a split-plot experimental design. Covering was considered as the main plot and planting system as the split-plot. We also tested two rootstocks [sour orange (*Citrus × aurantium*) and US-897 [‘Cleopatra’ mandarin (*Citrus reticulata*) × ‘Flying Dragon’ trifoliolate orange (*Poncirus trifoliata*)]]. Data were pooled for analysis purposes due to the lack of differences in the first year of cultivation.

TREES AND PLANTING METHODS. ‘Ray Ruby’ grapefruit trees on sour orange and US-897 rootstocks were purchased from licensed, certified

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.4047	acre(s)	ha	2.4711
0.3048	ft	m	3.2808
0.0929	ft ²	m ²	10.7639
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
6.4516	inch ²	cm ²	0.1550
0.4536	lb	kg	2.2046
0.001	ppm	g·kg ⁻¹	1000
1	ppm	mg·kg ⁻¹	1
6.8948	psi	kPa	0.1450
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

Fig. 1. Protected fresh grapefruit cultivation system for Asian citrus psyllid exclusion. At the center, four passively ventilated screen houses with 1/4-acre each (100 ft wide $\times$ 120 ft long $\times$ 14 ft tall). The service door is garage-style roll-up and measured 8 ft wide $\times$ 10 ft tall. A 12-ft wide $\times$ 12-ft long $\times$ 12-ft tall antechamber was built in 2015 to limit insect inclusion when the entrance door is opened. At the edges, four open-air plots; 1 acre = 0.4047 ha, 1 ft = 0.3048 m.

disease-free commercial nurseries [Sawmill Citrus Nursery (Fort Meade, FL) and Brite Leaf (Lake Panasoffkee, FL), respectively]. Trees were planted at a density of 792 trees/acre [spacing of 5.5 ft in-row and 10 ft between-row (eight trees/row and four rows on screen houses and eight trees/row and three rows on open-air, totaling 896 trees)]. The same tree density was used in all treatments. Potted trees were planted in 10-gal plastic containers (#10 Accelerator AP-10; Nursery Supplies, Chambersburg, PA). The plastic containers were filled with a medium consisting (v/v) of 50% clean, washed silica sand, 15% Florida peat-moss, 7.5% coconut fiber, 20% cypress sawdust, and 7.5% perlite (Harrell's, Lake Placid, FL). Plastic growing containers were placed on 16-inch² ceramic tiles to prevent tree roots from growing into the underlying native soil.

IRRIGATION. Each tree in this trial was serviced by two 2-gal/h flow drip emitters (SB-20; Bowsmith, Exeter, CA). Two weather stations (WatchDog 2900ET; Spectrum Technologies, Aurora, IL) were installed inside two of the screen houses, and an additional two stations were placed in two of the open-air plots. Trees grown in screen houses and open-air plots were watered to replenish the corresponding ET_o (mean of two weather stations) values for their respective growing environment. Monthly total and cumulative ET_o values for screen houses and open-air

plots are provided in Ferrarezi et al. (2017). From Jan. to July 2014, all trees automatically received daily irrigation volumes that were $\approx 33\%$ of the total ET_o value because of lower water demand. From July to Dec. 2014, trees received daily irrigation volumes that were 100% of the total ET_o . Trees were not irrigated on days where rainfall was equal to or greater than the ET_o .

FERTIGATION. We used 15N–2.6P–22.4K water-soluble fertilizer (Agrolution pHLow; Everris NA, Dublin, OH) with 15% total nitrogen [N (2.6% ammoniacal and 12.4% nitrate)], 2.6% phosphorus (P), 22.4% potassium (K), 3.3% calcium (Ca), 0.02% boron (B), 0.05% copper (Cu), 0.1% iron (Fe), 0.05% manganese (Mn), 0.0005% molybdenum (Mo), and 0.05% zinc (Zn). Fertilizer was mixed at a concentration of 150 lb fertilizer per 100 gal water in a 500-gal plastic stock tank plumbed in-line with the servicing irrigation system. A proportional 40 gal/min chemical injector (D8RE2; Dosatron International, Clearwater, FL) was installed directly upstream to the irrigation zone valves and connected to the fertigation stock tank. This injector added fertigation solution to each irrigation event and was adjusted seasonally to increase or decrease the proportional volume of fertigation solution added to the irrigation stream. The proportional injector's settings changed over time based on

nutritional needs by season, and the minimum, maximum, and annual mean of the proportioner (v/v) were as follows: 0.2% (February), 1.9% (September), and 0.8%. The screen houses and the open-air plots received the same amount of fertilizer throughout the study.

TREE CANOPY GROWTH PARAMETERS. Tree canopy growth parameters were measured monthly from Jan. to Dec. 2014. Eight trees were measured per screen house plot for each planting system and rootstock (total $n = 64$). Six trees were measured per open-air plot for each planting system and rootstock (total $n = 48$). Canopy diameters were measured in three directions (vertically and along the north–south and east–west lateral axes), and the three diameters averaged together to calculate the tree's canopy surface area (assumed shape was a sphere for surface area calculations).

LEAF AREA INDEX. LAI measurements were taken in June, July, Oct., Nov., and Dec. 2014. LAI for individual trees was calculated using a portable hemispherical camera and digital photograph analysis system (CI-110 Plant Canopy Imager; CID Bio-Science, Camas, WA). Only trees located within plots that contained a weather station were considered for further analysis. The LAI measurements were collected on a total of $n = 32$ trees for each in-ground and container plots on the screen houses, and on a total of $n = 24$ trees for each in-ground and container plots of the open-air controls. The camera system was placed on the ground beneath the target tree's canopy in the north–south direction and one image per tree was captured. An automated threshold algorithm (the “entropy crossover method”) included in the digital photograph analysis system's software was used to distinguish between leaves and sky background.

CWUE and LAIWUE were calculated by dividing individual tree LAI by the average daily ET_o value for each month of observation on each screen house. These calculations were made to determine the WUE of incremental growth of these tree responses.

CANOPY LIGHT INTERCEPTION. Measurements of canopy light interception were taken in June, July, Oct., Nov., and Dec. 2014. Light

interception was measured as the fraction of total radiation underneath the tree canopy relative to incident total radiation above the canopy (Oyarzun et al., 2007). Weather station measurements of total incident solar radiation above the tree canopies from inside the screen houses and the open-air plots were first converted to photosynthetic active radiation [PAR (400 to 700 nm)] units by multiplying solar radiation by a conversion factor of 4.57 (Thimijan and Heins, 1983). Next, the time-stamped PAR measurement from the hemispherical camera system's built-in ceptometer reading from underneath the tree canopy was matched with its corresponding value from the incident above-canopy measurements from the weather station in each growing environment and used to calculate monthly proportional PAR transmission.

LEAF INSTANTANEOUS PHOTOSYNTHETIC GAS EXCHANGE. Leaf water transpiration (E) and leaf VPD were recorded simultaneously on sun-exposed, asymptomatic, healthy leaves in June, July, Oct., Nov., and Dec. 2014. Leaf photosynthetic gas exchange was measured on one leaf from two trees for each combination of covering, planting system, and rootstock ($n = 16$). A portable photosynthetic gas exchange system (LI-6400XT; LI-COR Biosciences, Lincoln, NE) was used to take these measurements between 0900 and 1400 HR. A red–blue light emitting diode array provided illumination ($2000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR) within the sample chamber, and the reference carbon dioxide (CO_2) pressure was set to $400 \mu\text{mol CO}_2$ per mole of air.

LEAF NUTRITIONAL STATUS. Leaf samples were collected in June, July, Oct., Nov., and Dec. 2014. Six leaves per tree for eight trees per screen house on each planting system and rootstock, and six trees per open-air on each planting system and rootstock. Healthy, asymptomatic leaves on mature, hardened-off flushes were picked, washed in phosphate-free detergent, rinsed in distilled water, and placed into a drying oven at 50°C for 5–7 d. Dried leaves were sent to the UF/IFAS Analytical Services Laboratories in Gainesville, FL, for the determination of leaf N, Mg, Mn, Zn, and Fe.

Fig. 2. ‘Ray Ruby’ grapefruit (A) canopy surface area, (B) leaf area index, and (C) canopy light interception as photosynthetic active radiation (PAR) transmission after the first year of transplant under different coverings [enclosed screen houses and open-air (control)] and planting methods (in-ground and container-grown). The purpose of screen houses was the exclusion of asian citrus psyllid. Data are mean $\pm$ SE. Data were analyzed with a linear mixed-effect repeated measures model. Tukey-adjusted multiple comparison tests were used to separate treatment means for the significant effect. Means with different lowercase letters indicate statistically significant differences for the given month ($P \leq 0.05$); $1 \text{ m}^2 = 10.7639 \text{ ft}^2$.

STATISTICAL ANALYSIS. All subsamples were averaged to obtain a single mean for each replicated experimental unit before subsequent analyses. The number of replicated experimental units for all combinations of “covering” and “planting” effects (rootstocks were pooled for analysis purposes due to the lack of differences in the first year of

cultivation) for the observed tree responses are tree canopy surface area measurements ($n = 8$), LAI, PAR transmission, and incremental growth efficiency measurements ($n = 4$), leaf photosynthetic gas exchange measurements ($n = 8$), and leaf nutritional status ($n = 8$). All measured tree responses were evaluated using a linear mixed-effect repeated measures

model, implemented using the “lme” function library (Pinheiro and Bates, 2000) in the R computing environment (version 3.3.3; R Foundation, Vienna, Austria). In the repeated measures models, the main effects “covering”, “planting”, “date”, and all of their combinatory interaction effects were evaluated simultaneously. A compound-symmetry correlation structure was added to these repeated measures models to account for serial observations. Significant ($P \leq 0.05$) model effects were subsequently evaluated with Tukey-adjusted multiple comparison tests to separate treatment means, where necessary, using the “multcomp” function library in R.

Replicate means of CWUE were regressed on leaf VPD measurements using values from June, July, and Oct. 2014, using a dataset consisting of both coverings (enclosed screen houses and open-air plots, $n = 12$, each). Nonlinear regression was used to evaluate this relationship, implemented using the “nls” package library in R.

Results

Tree canopy surface area increased from Jan. to Dec. 2014 (Fig. 2A). Young grapefruit trees grown inside the screen houses (with in-ground and container-grown plots averaged together) had larger canopy surface areas compared with trees grown in the open-air plots, from July to Dec. 2014 [“covering” × “date”; $P < 0.01$ (Fig. 2A)].

Tree LAI was measured in June, July, and from Oct. to Dec. 2014 (Fig. 2B). Trees inside the screen houses and planted in-ground had the largest mean LAI (‘covering’ × ‘planting’ × ‘date’; $P = 0.02$). Trees container-grown inside the screen house plots had intermediate LAI, whereas trees grown in the open-air plots (in-ground and container-grown) had the smallest LAI from June to Dec. 2014 (Fig. 2B).

Tree canopy proportional *PAR* transmission decreased throughout the year from June to Dec. 2014 (Fig. 2C). The time-averaged proportional *PAR* transmission of young trees grown inside the screen houses and in-ground were lower than the open-air plots (‘covering’ × ‘planting’; $P = 0.04$). The values for the container-grown trees inside the screen houses were intermediate to those of in-ground grown trees inside and the open-air plots (Fig. 2C).

Monthly CWUE increased from Mar. to Dec. 2014, for all treatments, especially from July to December (Fig. 3A). The canopy area and LAI per unit of ET_o of in-ground trees within the screen houses were larger than other treatments (‘covering’ × ‘planting’ × ‘date’; $P < 0.01$). Trees grown in the open-air plots (in-ground and container-grown) showed the least canopy growth per unit of ET_o (Fig. 3A). LAI water use efficiency increased from June to Dec. 2014 (Fig. 3B). Trees grown in the open-air plots (in-ground and container-grown) developed the least LAI per unit of ET_o , with container-grown trees inside having intermediate values (‘covering’ × ‘planting’ × ‘date’; $P = 0.02$). This response was caused by the limitation

in water and nutrient supply because of container-grown citrus production, reducing LAI in December (Fig. 3B).

Instantaneous measurements of leaf *E* and VPD were taken in June, July, and from Oct. to Dec. 2014 (Fig. 4). Time-averaged leaf *E* values for in-ground trees inside the screen houses were larger than the container-grown trees (inside screen houses and open-air plots), and trees grown in-ground in the open-air plots had intermediate values to the other treatments [‘covering’ × ‘planting’; $P = 0.03$ (Fig. 4A)]. Time-averaged leaf VPD values for trees grown within the screen houses were smaller than those in the open-air plots [‘covering’ and ‘date’; $P < 0.01$ (Fig. 4B)]. Monthly (June, July, and Oct.) values

Fig. 3. ‘Ray Ruby’ grapefruit (A) canopy surface area water use efficiency (CWUE) and (B) leaf area index water use efficiency (LAIWUE) after the first year of transplant under different coverings [enclosed screen houses and open-air (control)] and planting methods (in-ground and container-grown). The purpose of screen houses was the exclusion of asian citrus psyllid. Data are mean $\pm$ SE. Data were analyzed with a linear mixed-effect repeated measures model. Tukey-adjusted multiple comparison tests were used to separate treatment means for the significant effect. Means with different lowercase letters indicate statistically significant differences for the given month ($P \leq 0.05$); $1 \text{ m}^2 \cdot \text{mm}^{-1} = 273.4033 \text{ ft}^2/\text{inch}$, $1 \text{ m}^2 \cdot \text{m}^{-2} \cdot \text{mm}^{-1} = 25.4000 \text{ ft}^2/\text{ft}^2 \text{ per inch}$.

Fig. 4. ‘Ray Ruby’ grapefruit (A) leaf transpiration (E) and (B) leaf vapor pressure deficit (VPD) after the first year of transplant under different coverings [enclosed screen houses and open-air (control)] and planting methods (in-ground and container-grown). The purpose of screen houses was the exclusion of asian citrus psyllid. Data are mean $\pm$ SE. Data were analyzed with a linear mixed-effect repeated measures model. Tukey-adjusted multiple comparison tests were used to separate treatment means for the significant effect. Means with different lowercase letters indicate statistically significant differences for the given month ($P \leq 0.05$); 1 kPa = 0.1450 psi.

of CWUE were negatively and non-linearly related to leaf VPD measurements (Fig. 5).

Container-grown grapefruit trees in the open-air plots had higher leaf N concentrations in all months, except July and October, than the in-ground grown trees in both cultivation environments [“covering” $\times$ “planting” $\times$ “date”; $P < 0.01$ (Fig. 6A)]. Leaf Mg declined steadily in all treatments from June to December [“covering” $\times$ “date”; $P < 0.01$ (Fig. 6B)]. Leaf Mg differed in June and July; thereafter, leaves from trees grown in the open-air plots had greater leaf Mg concentrations than leaves collected from inside the screen houses (Fig. 6B).

Leaf Mn and Zn concentrations were greater from the open-air plots, compared with the screen houses in June and July [“covering” $\times$ “date”; $P < 0.01$ (Fig. 6C and D)]. These

differences were not observed from October to December (Fig. 6C and D). Leaf Fe concentration varied throughout the growing season from June to December for all treatments [“date”; $P < 0.01$ (Fig. 6E)].

Discussion

Grapefruit trees grown in-ground inside the protective screen houses developed more canopy surface area and greater LAI than container-grown trees in the screen houses and for both planting systems in the open-air. Mechanisms responsible for this response are related to increased CWUE and LAIWUE and reduced leaf VPD. Increases in productivity when radiation loads are moderately reduced have been reported previously for young citrus trees (Raveh et al., 2003).

Canopy architecture is an important component in light interception

for tree crops. As tree canopy and LAI increased, the proportion of PAR intercepted by photosynthetically active canopy elements also increased. This relationship is often described by biophysical models derived from the Lambert–Beer law (Annandale et al., 2004; Oyarzun et al., 2007, 2011). In general, PAR interception by a fruit tree foliage increases exponentially with increasing canopy width and LAI. In the current study, grapefruit trees grown in-ground and inside the screen houses transmitted less PAR through their canopies compared with trees grown in the open-air plots. This is despite the observation that $\approx 23\%$ less total solar radiation incident reached tree canopies within the screen enclosures, compared with the open-air plantings (Ferrarezi et al., 2017). The reduction in proportional PAR transmittance of trees grown inside the screen houses is likely attributed to their larger canopy areas (Fig. 2A) and LAI values (Fig. 2B). Responses of canopy area and LAI of grapefruit trees grown inside screen houses are similar to Cohen et al. (2005), who also erected shade-cloth over grapefruit plantings.

Tree CWUE and LAIWUE were greater inside the screen houses when compared with the open-air plots (Fig. 3). Cumulative values of ET_o inside the screen houses were 21% lower than the open-air plots during the same time period (Ferrarezi et al., 2017). Greater WUE is likely a combination of increased canopy growth and LAI as well as reduced evaporative water losses within the completely enclosed screen houses relative to the open-air plots. Glenn (2010) noted that leaf WUE decreased in apple trees shaded with an artificial particle film because of increased stomatal conductance (g_s) and increased photosynthetic net CO_2 assimilation (P_N). The author suggested that particle film-coated apple leaves had higher rates of P_N , relative to nontreated controls because of diffusive light scattering caused by the particle film and that the consequent increases in P_N led to increases in leaf water loss. This feedback loop is also evident in reports published by Cohen et al. (2005) and Jifon and Syvertsen (2003), whereby citrus trees covered with shade-cloth had increased leaf rates of P_N and g_s . Results obtained in the current study are in agreement with these previous

Fig. 5. Relationship between 'Ray Ruby' grapefruit tree mean canopy surface area water use efficiency (CWUE) and leaf vapor pressure deficit (VPD). Data are treatment plot means from June, July, and Oct. 2014, for open-air plots (○) and antipsyllid screen houses (●) (each growing environment, $n = 12$). The purpose of screen houses was the exclusion of asian citrus psyllid. Dashed line represents model fitted values; 1 kPa = 0.1450 psi, 1 $\text{m}^2 \cdot \text{mm}^{-1}$ = 273.4033 ft^2/inch .

reports. Cohen et al. (2005) also noted the importance of scattered diffusive light in leaf P_N increase. The proportion of scattered diffusive PAR within the enclosed screen houses was not quantified in the current investigations but anecdotal observations suggest that the screen material used leads to greater scattering of incoming PAR compared with the open-air plots. Scattered, diffusive PAR is an important component in modeling tree orchard light interception (Oyarzun et al., 2007) and should be included in any future refinements and simulation efforts related to a cultivation system as the one described in the current study.

Citrus leaf VPD and temperature are reduced in shaded environments. Jifon and Syvertsen (2003) covered sweet orange (*Citrus sinensis*) and grapefruit trees underneath reflective, aluminized shade cloth, and observed that leaf VPD and leaf temperature values were reduced in trees grown throughout the course of the day. Similarly, Cohen et al. (2005) found that shade cloth reduced grapefruit leaf temperatures relative to open-air plantings. In the current investigation, the time-averaged leaf VPD measurements were lower on trees grown in-ground inside the screen houses compared with the open-air

plots. Jifon and Syvertsen (2003) also observed a negative relationship between citrus leaf g_s and leaf VPD, implying that leaf water status decreases with increasing leaf VPD. This is in accordance with this study, where time-averaged leaf E values were greater for trees grown inside the screen houses.

The larger grapefruit canopy of trees growing inside the enclosed screen houses appears to indicate that increased vegetative growth (Fig. 2A) is facilitated by lower environmental evaporative demand (Fig. 3) because of higher g_s compared with plants grown in open-air plots. The higher VPD outside the greenhouse closed stomata and reduced P_N (data not shown). Increases in leaf VPD were associated with decreases in leaf P_N , g_s , and WUE when shade cloth was used on young grapefruit trees (Jifon and Syvertsen, 2003). Decreases in plant WUE with increasing VPD has also been observed in open-air olive (*Olea europaea*) orchards, and the underlying mechanism was described as a co-occurring reduction in leaf water vapor loss and increased resistance to CO_2 entering substomatal chambers (Testi et al., 2008). Similar results were also reported by Ouyang et al. (2013) for peach (*Prunus persica*) tree canopies. According to

Jifon and Syvertsen (2003), Ouyang et al. (2013) and Testi et al. (2008), WUE declined as VPD increased more than 2 kPa. These observations agree with the results obtained in the current investigation where time-averaged leaf VPD values from trees grown inside the screen houses were lower compared with the open-air plots (Fig. 4B). In addition, CWUE was negatively related to leaf VPD values (Fig. 5).

Container-grown grapefruit trees developed less canopy area, either inside enclosed screen houses or in the open-air plots, compared with trees grown in-ground in their respective covering treatments. In addition to developing smaller canopies, container-grown trees in the open-air plots had consistently higher leaf N concentrations throughout the study period compared with the other treatments (Fig. 6A). Results from the current study are similar to Ran et al. (1992), who cultivated peach trees in containers, and found container-grown plants showed lower dry weight and higher N concentrations. Trees grown in the open-air plots received more fertigation solution throughout the study period relative to the trees grown inside the enclosed screen houses. The fertigation issues in the current study were based on the growing environment's ET_0 values. The open-air plots experienced an average of 21% greater ET_0 values, compared with the growing environment inside the screen houses (Ferrarezi et al., 2017), over the period of study. Therefore, $\approx 21\%$ more fertigation solution was also delivered to grapefruit trees in the open-air plots. However, in the open-air plots, container-grown trees had greater leaf N concentrations compared with the trees grown in-ground as well (Fig. 6A). Ran et al. (1992) reported that nitrate-N concentrations in peach trees' transpiration were elevated in smaller volume containers because there was a greater number of small diameter, fine roots in smaller containers. Fine root abundance was not quantified in the current study, but authors have visually noticed an abundance of small roots filling the plastic growing containers. Nevertheless, leaf N concentrations of all treatments in the current study met or exceeded the "optimum" concentration (25–27 $\text{g} \cdot \text{kg}^{-1}$) recommended

Fig. 6. ‘Ray Ruby’ grapefruit leaf (A) nitrogen (N), (B) magnesium (Mg), (C) manganese (Mn), (D) zinc (Zn), and (E) iron (Fe) after the first year of transplant under different coverings [enclosed screen houses and open-air (control)] and planting methods (in-ground and container-grown). The purpose of screen houses was the exclusion of asian citrus psyllid. Data are mean $\pm$ SE. Data were analyzed with a linear mixed-effect repeated measures model. Tukey-adjusted multiple comparison tests were used to separate treatment means for the significant effect. Means with different lowercase letters indicate statistically significant differences for the given month ($P \leq 0.05$); $1 \text{ g}\cdot\text{kg}^{-1} = 1000 \text{ ppm}$, $1 \text{ mg}\cdot\text{kg}^{-1} = 1 \text{ ppm}$.

by the UF/IFAS Extension Service (Koo et al., 1984). The only time that this “optimum” level was not reached was for the in-ground grown trees cultivated within the screen houses in July. Rainfall in June was the second-highest monthly precipitation rate in 2014 for this study (Ferrarezi et al., 2017), and explains the lower leaf N concentrations in the following month of July; fertigation was not applied for most of June because rainfall was equal to or greater than observed ET_o values. Thus, July leaf N measurements were lower because of the lower amount of fertilizer applied in June.

Leaf concentrations of Mg, Mn, and Zn were higher in June and July in the open-air plots compared with leaves collected from within the screen houses (Fig. 5B–D). This is likely due the difference in ET_o values between the two different coverings; trees grown in the open-air plots received more fertigation solution because of the effort to replenish the greater ET_o . However, by October, ET_o values in both environments began to decline and converge toward the end of the year, resulting in both growing environments receiving less soluble fertilizer. The “suboptimum” concentrations observed for leaf Mg and Zn ($3\text{--}4.9$ and $25\text{--}100 \text{ mg}\cdot\text{kg}^{-1}$, respectively) (Koo et al., 1984) are also likely related to absence (Mg) and low concentration (Zn) in the soluble fertilizer used in this study. However, leaf Mn concentrations in the current study exceeded recommended levels ($25\text{--}100 \text{ mg}\cdot\text{kg}^{-1}$) (Koo et al., 1984). Supplemental Mg and Zn applications through the fertigation system later in the year might help to maintain the minerals within recommended ranges.

Conclusion

Trees grown inside the enclosed screen houses had higher canopy surface area, LAI, CWUE and LAIWUE compared with the open-air plots. The increase in plant WUE inside the screen houses were linked to greater leaf E and leaf VPD. Grapefruit trees grown in the open-air plots had greater PAR canopy transmission compared with the trees growing inside the screen houses. While trees grown inside experienced less incident PAR light, they intercepted a larger proportion of PAR . Trees planted in-ground and inside the

screen houses developed the largest canopies, the most leaves, and higher WUE. Growing young grapefruit trees in-ground within antipsyllid screen houses may offer the benefit of rapidly bringing trees into production that remain HLB-free. The ultimate benefit of growing citrus trees in the screen houses would be increased yield and fruit quality, which may or may not be well correlated with tree growth. Therefore, it is still too early to conclude if the trees grown in the screen house will be superior to trees grown in the open-air.

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