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Effect of Individual Protective Covers on Young 'Valencia' Orange (*Citrus sinensis*) Tree Physiology

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Abstract. **Huanglongbing (HLB), an important citrus disease, causes many physiological and anatomical changes such as phloem dysfunction, imbalance in carbohydrate partitioning, decrease in leaf chlorophyll, and nutritional imbalances in the affected trees, ultimately resulting in tree decline. In Florida, HLB is associated with phloem-limited bacteria *Candidatus* Liberibacter asiaticus (*C*Las), and it is vectored by the Asian citrus psyllid (*Diaphorina citri*). No cure for HLB has been found, and most of the HLB management efforts have been focused on vector control or exclusion, improved nutrient management, and the use of HLB-tolerant rootstocks. Individual protective covers (IPCs) are a type of psyllid exclusion tool that is increasingly used by growers for HLB management of newly planted citrus trees. However, no studies have evaluated their influence on citrus tree physiology. This study investigated the effect of IPCs and different rates of insecticides on *C*Las infection and different physiological attributes, including soluble (glucose, fructose, and sucrose) and nonsoluble (starch) carbohydrates, leaf chlorophyll, and leaf macronutrients and micronutrients over 2.5 years of field growth. The treatments (tree cover and insecticides rate) were applied in newly planted 'Valencia' sweet orange (*Citrus sinensis*) trees grafted on 'Cleopatra' (*C. reticulata*) rootstock. The IPCs prevented *C*Las transmission and accumulation of foliar starch, sucrose, and glucose commonly associated with HLB. IPC-covered trees had more leaf chlorophyll-a and chlorophyll-b than noncovered trees and more leaf nitrogen (N) and zinc (Zn). Our findings suggest that IPCs effectively prevent *C*Las infection and maintain the physiological health of young citrus trees under heavy HLB pressure. Therefore, IPCs are recommended as an important component of integrated pest management for this devastating disease.**

Huanglongbing (HLB) disease, also known as citrus greening, has affected the citrus industry worldwide, and most of the commercially important scion cultivars are susceptible (Bove 2006; Dala-Paula et al. 2019). The disease was first reported in southern China in 1919, and it was named "Huang-long-bing" by local farmers, which means "yellow dragon" or "yellow shoot disease." In Florida, the disease is associated with the phloem-limited bacterium *Candidatus* Liberibacter asiaticus (*C*Las) and is transmitted by Asian citrus psyllids (ACPs) *Diaphorina citri*. Although ACPs were found in Florida in 1998, the first incidence of HLB was not reported until 2005 (Bove 2006; Halbert 2005; Halbert and Manjunath 2004). Since the discovery of HLB in Florida, the citrus industry has been severely affected, resulting in major reductions in both citrus acreage and yield (US Department of Agriculture, National Agricultural Statistics Service 2019). Citrus canker and major hurricanes in 2017 and 2022 further exacerbated the decline of a formerly iconic industry (de Carvalho et al. 2021; Shahbaz et al. 2023).

No known cure for HLB has been found. When trees become infected, the disease is difficult to manage (Bove 2006; Gaire et al. 2022; Gottwald 2010; Halbert and Manjunath 2004). Several management practices such as vector control through insecticide use, improved nutrient management, and the use of HLB-tolerant rootstocks have been used to maintain the sustainability of citrus under HLB-endemic conditions (Bowman and Albrecht 2020; Bowman et al. 2016; Kunwar et al. 2021, 2023; Rodrigues et al. 2020; Stansly et al. 2014). Removal of infected trees from existing citrus groves and the use of disease-free plant material in new plantings have been recommended (Boina and Bloomquist 2015; Halbert and Manjunath 2004), but they are not practical in areas where HLB is endemic, such as in Florida (Graham et al. 2020), because most of the trees are infected. Trunk injection of oxytetracycline was recently reported to improve tree health and productivity of HLB-affected citrus trees (Archer et al. 2022a, 2022b), and a local special need label now allows the use of this methodology to manage HLB in Florida. However, this technology is not suitable for newly planted trees, which are most vulnerable to infection with *C*Las (Hall et al. 2016; Rogers et al. 2012). Preventing infection during the early growth stage is essential because, otherwise, trees will never become productive (Chung and Brlansky 2005).

HLB causes many anatomical and physiological disruptions in affected trees. *C*Las are localized in the sieve tubes of the phloem, which are rich in nutrients supporting bacterial growth. Previous evidence indicates that *C*Las-infected phloem tissue undergoes major anatomical changes such as sieve element plugging, resulting in phloem dysfunction and, ultimately, in phloem necrosis (Achor et al. 2010; Deng et al. 2019; Kim et al. 2009; Koh et al. 2012). Disruption of phloem tissue inhibits the transport of photoassimilates from source to sink organs, leading to starch accumulation in the leaves (Achor et al. 2010; Koh et al. 2012). Upregulation of important starch biosynthesis enzymes, such as ADP-glucose pyrophosphorylase, starch synthase, granule-bound starch synthase, and starch debranching enzyme, and downregulation of starch degradation enzymes, such as beta-amylase, have also been confirmed in response to *C*Las infection, contributing to leaf starch accumulation (Albrecht and Bowman 2008; Kim et al. 2009). A study by Schaffer et al. (1986) of citrus showed that starch accumulations can cause the disintegration of the chloroplast thylakoid system and decrease leaf chlorophyll levels. Downregulation of photosynthesis and chlorophyll-associated genes such as chlorophyll a–b binding family protein/early light-induced protein and photosystem II 5 kDa protein in response to *C*Las infection has also been observed (Albrecht and Bowman 2008). Imbalance in carbohydrate partitioning and disintegration of the chloroplast thylakoid system are believed to produce foliar HLB symptoms such as blotchy mottle and chlorosis (Achor et al. 2010; Schaffer et al. 1986; Schneider 1968). This imbalance in carbohydrate partitioning is one of the main reasons for the steady decline of HLB-affected trees (Etxeberria et al. 2009).

It has been suggested that starch accumulation in the leaves of HLB-affected trees concurs with starch depletion in the roots (Aritua et al. 2013; Etxeberria et al. 2009). Deprivation of photosynthates in the roots along with disruption in phloem function cause fibrous root decline of HLB-affected trees (Johnson et al. 2014). This restricts the uptake and translocation of water and nutrients, ultimately causing nutritional disorders and metabolic imbalances attributable to nutrient depletion or interference with transportation (Kumar et al. 2018; Mattos et al. 2020; Medina et al. 2014; Spann and Schumann 2009).

Under the present HLB-endemic conditions in Florida (Graham et al. 2020), it is critical to prevent *C*Las transmission to new plantings and keep them disease-free and productive. Individual protective covers (IPCs) are a type of psyllid exclusion tool designed to prevent the transmission of HLB in young citrus trees (Alferez et al. 2021; Gaire et al. 2022). IPCs have gained the attention of Florida citrus growers in recent years and are being increasingly used in commercial citrus production. It has been proven that vector exclusion is an

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effective management approach for diseases vectored by insects in citrus and other crops (Berlinger et al. 2002; Polston and Lapidot 2007; Schumann et al. 2021; Singh et al. 2006). For example, the citrus under protective screen has been successfully used for the exclusion of psyllids and HLB prevention in citrus (Ferrarezi et al. 2019; Schuman et al. 2021), Nylon mesh screens were effective for the control of the leaf curl virus in sweet pepper (Singh et al. 2006), and 50-mesh screens were effective for the control of yellow leaf curl disease in tomato (Berlinger et al. 2002; Polston and Lapidot 2007).

Depending on the screen material, mesh screens can modify microclimatic conditions and affect the physiology and health of plants in response to the shading induced by their use (Budiarto et al. 2019; Haijun et al. 2015; Mahmood et al. 2018). Moderate shading has been found to increase foliar chlorophyll concentrations in citrus (Budiarto et al. 2019; Incesu et al. 2014). The lower evaporative demand under shaded conditions can improve stomatal conductance and, hence, carbon dioxide assimilation and water use efficiency (Haijun et al. 2015). An improved photosynthetic rate has also been reported in response to artificial shading induced by black net screens for citrus (Budiarto et al. 2019). A recent study of a feral citrus population reported the positive effects of shading to promote photosynthetic activity and mitigate the severity of HLB (Vincent et al. 2021). We have previously shown that IPCs can reduce vapor pressure deficits (Gaire et al. 2022). However, relatively little is known about the effects of IPCs, which are composed of a white high-density polyethylene mesh, on the physiology of young citrus trees.

We hypothesized that the microclimatic modifications induced by IPCs coupled with a disease-free plant system can be advantageous for the physiological performance of the tree and, therefore, overall tree growth and productivity. The beneficial effects of IPCs on vector exclusion and tree growth were previously reported (Gaire et al. 2022). The objective of this study was to evaluate the physiological attributes of 'Valencia' (*Citrus sinensis*) scion grafted on 'Cleopatra' (*C. reticulata*) rootstock with and without IPCs and the interactions with different rates of insecticides.

Materials and Methods

Plant material and study site

The experiment started with the planting of new certified disease-free citrus trees composed of 'Valencia' (*Citrus sinensis*) scion grafted on 'Cleopatra' (*C. reticulata*) rootstock that were obtained from a commercial registered citrus nursery (Southern Citrus Nurseries, Dundee, FL, USA). Ninety trees were planted in January 2018 at the Southwest Florida Research and Education Center (SWFREC) research farm in Immokalee, Collier County, FL (26°27′51.4″N, 81°26′39.9″W). The soil type at this location is a sandy spodosol with little organic matter and low cation exchange capacity (Mylavarapu et al. 2016; Pokhrel et al. 2020). Sand, silt, and clay concentrations determined at the start of the experiment were 96.7%, 1.2%, and 21%, respectively; organic matter, pH, and cation exchange capacity were 0.7%, 7.9, and 7.6 mEq/100 g, respectively.

Experimental design

The experiment was arranged in a completely randomized 2 × 3 factorial design. The first factor (tree cover) had two levels: IPC or no IPC. The second factor (insecticide rate) had three levels: full recommended rate (full), half the recommended rate (half), and no insecticides (zero). A total of six treatment interactions were replicated five times; each replication consisted of linear plots of three trees. Trees were planted in six rows of 15 trees each at a spacing of 8 feet (2.4 m) within rows and 22 feet (6.7 m) between rows.

Treatments

IPCs (Tree defender Inc., Dundee, FL, USA) were installed on young citrus trees at the time of planting (Jan 2018). IPCs made of monofilament high-density polyethylene with a mesh size of 50 (50 holes per linear inch, 0.297-mm holes) were used for the experiment (Fig. 1). The mesh had pores smaller than the average width of psyllid adults (≈0.6 mm) and were expected to prevent access of psyllids to the tree canopy (Ebert et al. 2021). Four-foot-tall (1.2 m) IPCs were installed on tree plots immediately after planting. After 18 months, the 4-foot IPCs were replaced by 7-foot-tall (2.1 m) IPCs to accommodate the expanded tree canopy and allow further expansion. The 7-foot IPCs remained on the trees for an additional 12 months until removal in Aug 2020. IPCs were tied with zip ties at the base of the trunk to prevent psyllids and other insects from entering.

Insecticide treatments followed the University of Florida's Institute of Food and Agricultural Sciences guidelines for young trees (Rogers 2014) and consisted of rotations of the systemic neonicotinoids, imidacloprid (40.4% a.i.; Nuprid 4F Max; Nufarm, Alsip, IL, USA), clothianidin (23% a.i.; Belay; Valent, Walnut Creek, CA, USA), and thiamethoxam (75% a.i.; Platinum 75 SG; Syngenta, Wilmington, DE, USA). The rate and time of insecticide application were adjusted based on tree age and are summarized in Supplemental Tables 1 and 2 (Rogers 2014). Insecticides were diluted in water, and each tree received a soil drench of 300 mL material per application. Trees were irrigated three times per week by under-tree microjets. Diamond-R 8–8–8 young tree blend (Diamond R Fertilizer, Fort Pierce, FL, USA) was applied at the rate of 0.5 lb (227 g) per tree in year 1, and at a rate of 1.0 lb (454 g) per tree in years 2 and 3. Diamond R CitriBlend 12–8–6 control release fertilizer (Diamond R Fertilizer, Fort Pierce, FL, USA) was applied at a rate of 0.5 lb (227 g) per tree in years 1 to 3. Weeds were managed as needed using standard practices.

HLB disease assessment and CLas detection

The HLB disease assessment and quantitative real-time polymerase chain reaction for *C*Las detection in the leaves were performed as outlined in the study by Gaire et al. (2022). Samples were taken during Spring and Summer of 2019 and 2020.

Soluble and nonsoluble carbohydrate analysis

Extraction. The soluble (glucose, fructose, and sucrose) and nonsoluble (starch) carbohydrates in leaves were determined using enzymatic assays. Leaves were collected during Summer (Jul) 2019, Spring (Mar) 2020, and Summer (Jul) 2020. Three to four mature fully expanded leaves from recent flushes were collected from the middle tree in each three-tree plot. Leaves were pulverized in liquid nitrogen with a mortar and pestle, and 150 mg of ground tissue was used for carbohydrate extraction. Each sample was extracted twice in 1 mL of 80% ethanol for 1 h at 70 °C and centrifuged for 5 min at 20,000 g_n. Supernatants were combined for the analysis of soluble carbohydrates. Insoluble pellets were used for starch determination.

Soluble carbohydrate determination. Supernatants were dried in an Eppendorf vacufuge concentrator (Thermo Fisher Scientific, Waltham,

Fig. 1. Citrus tree with an individual protective cover and tree wrap.

MA, USA), resuspended in 500 μL of ultrapure water, and centrifuged for 5 min at 20,000 g_n. Supernatants were used for soluble carbohydrate determination. Glucose and fructose were measured sequentially by the enzymatic assay as described in the study by Gomez et al. (2007). In brief, glucose determination was based on the phosphorylation of glucose to form glucose-6-phosphate (G6P) by hexokinase (HK), followed by the conversion of G6P and NAD to gluconate-6-phosphate (6PGlcU) and NADH by glucose-6-phosphate dehydrogenase (G6PDH). Fructose determination involved the phosphorylation of fructose to form fructose-6-phosphate (F6P) by HK, the conversion of F6P to G6P by phosphor-glucose isomerase, and the conversion of G6P and NAD to 6PGlcU and NADH. NADH production was measured at 340 nm using a microplate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). Sucrose was determined indirectly in 10-fold diluted supernatants by measuring glucose as described after cleavage into glucose and fructose by invertase. All enzymatic reactions were performed in 50 mM triethanolamine-HCl, 5 mM MgSO4, 0.02% bovine serum albumin, and 0.5 mM dithiothreitol. Assays were performed at least in duplicate. Soluble carbohydrate contents were expressed as μg/mg leaf tissue.

Starch determination. Pellets remaining from soluble sugar extraction were dried using an Eppendorf vacufuge concentrator (Thermo Fisher Scientific, Waltham, MA, USA) and resuspended in 900 μL of ultrapure water. Starch was dispersed by autoclaving for 1 h at 121 °C and 19 psi. An equal volume of sodium acetate buffer (0.1 M, pH 4.65) was added together with 5 μL (14 units) of amyloglucosidase (Sigma-Aldrich, St. Louis, MO, USA), and samples were incubated for 100 min at 56 °C. After centrifugation for 5 min at 20,000 g_n, supernatants were diluted 10-fold and used for starch determination. Starch was measured indirectly by enzymatic assay of released glucose as described for soluble carbohydrates and expressed as μg/mg leaf tissue. Foliar starch content was correlated with cycle threshold values obtained from quantitative real-time polymerase chain reaction during Summer (Jul) 2019, Spring (Mar) 2020, and Summer (Jul) 2020.

Leaf chlorophyll analysis

Chlorophyll was measured using the method described by Nayek et al. (2014) with some modifications. The same leaves collected for the carbohydrates analysis were used for the leaf chlorophyll analysis during Summer (Jul) 2019, Spring (Mar) 2020, and Summer (Jul) 2020. Leaves were pulverized under liquid nitrogen, and 100 mg of tissue was extracted in 2 mL of 95% ethanol. Extracts were centrifuged at 20,000 g_n for 15 min at 4 °C, and supernatants were used for the analysis. Absorbances were measured at 664 nm and 649 nm using a spectrophotometer (Molecular Devices, CA, USA). Chlorophyll a, chlorophyll b, and total chlorophyll contents were calculated using the following equations:

$$\text{Chlorophyll a} = 13.36 \times \text{A664} - 5.19 \times \text{A649} \quad [1]$$

$$\text{Chlorophyll b} = 13.36 \times \text{A664} - 5.19 \times \text{A649} \quad [2]$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b} = 22.24 \times \text{A649} + 5.24 \times \text{A664} \quad [3]$$

where A664 = absorbance at 664 nm and A649 = absorbance at 649 nm.

The ratio of chlorophyll a to chlorophyll b (chl a/b) was also calculated.

Leaf nutrient analysis

Leaf nutrients were analyzed at the end of the study period [Summer (Aug) 2020]. Mature leaves from the recent flush were randomly collected from each tree and pooled within each plot for a total of 30 leaves per sample. Macronutrients, nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S), and micronutrients, boron (B), zinc (Zn), manganese (Mn), Iron (Fe), and copper (Cu), were analyzed by Waters Agricultural Laboratories, Inc. (Camilla, GA, USA). Furthermore, N was determined by the combustion method as described by Sweeney (1989). Inductively coupled argon plasma atomic emission (ICAP) spectrometry was used to determine the other macronutrients and micronutrients after digesting leaves with nitric acid and hydrogen peroxide solution (Havlin and Soltanpour 1980; Huang and Schulte 1985).

Statistical analysis

An analysis of variance was conducted to determine the effects of tree cover, insecticide rate, and their interaction for all variables using a general linear model and R programming (version 4.0.3; The R Foundation 2020). Mean separation was performed using Tukey's honestly significant difference test. Pearson's correlation coefficients were calculated for selected response variables. Differences were defined as statistically significant when $P < 0.05$.

Results

*C*Las detection

From Spring 2019 to Summer 2020, the cycle threshold values of *C*Las in leaf tissues ranged from 38.9 to 40.7 for trees with IPCs, confirming that trees were free of *C*Las during the study period. The cycle threshold values of trees without IPCs ranged from 21.1 to 28.0, indicating that they were infected with *C*Las (Gaire et al. 2022).

Soluble and nonsoluble carbohydrates

The leaf glucose content varied significantly between trees with and without IPCs during Summer 2020, but not during Summer 2019 or Spring 2020 (Table 1). During Summer 2020, trees with IPCs had significantly less glucose (1.2 μg/mg) compared with trees without IPCs (1.8 μg/mg). No significant effects of the insecticide rate and tree cover × insecticide rate interaction were found for glucose.

The leaf fructose content ranged from 0.7 μg/mg to 1.2 μg/mg throughout the study period, but there was no significant difference between covered and noncovered trees (Table 1). There were no significant effects of the tree cover, insecticide rate, or interaction between both factors.

The leaf sucrose content varied significantly between trees with and without IPCs during Summer 2019 and 2020, but not during Spring 2020 (Table 1). Trees covered with IPCs had significantly less sucrose (16.3 μg/mg and 21.5 μg/mg, respectively) compared with noncovered trees (21.3 μg/mg and 28.4 μg/mg, respectively) during Summer 2019 and Summer 2020, respectively. The insecticide rate and the tree cover × insecticide rate interaction were not significant for sucrose.

The leaf starch content was significantly influenced by tree cover at all three time points. The least starch was measured for trees with IPCs (12.0–23.2 μg/mg), and the most starch was found in trees without IPCs (44.2–80.7 μg/mg) (Table 1). There were no significant effects of the insecticide rate and the tree cover × insecticide rate interaction on starch.

There was a significant negative correlation between cycle threshold values and starch content (correlation coefficients: R = −0.85, R = −0.66, and R = −0.70 during Summer 2019, Spring 2020, and Summer 2020, respectively) (Fig. 2).

Leaf chlorophyll

Chlorophyll a, chlorophyll b, and the total chlorophyll content were significantly influenced by tree cover (Table 2). The chlorophyll a content was significantly higher in trees covered with IPCs (3.1–5.1 μg/mg) compared with uncovered trees (2.0–4.2 μg/mg) in Summer 2019, Spring 2020, and Summer 2020. The chlorophyll b content was significantly higher in trees with IPCs (1.0 and 1.8 μg/mg, respectively) compared with trees without IPCs (0.6 and 1.4 μg/mg, respectively) during Summer 2019 and Summer 2020, but not during Spring 2020. The total chlorophyll content was also significantly higher in trees with IPCs (4.2–6.9 μg/mg) compared with trees without IPCs (2.6–5.5 μg/mg) during Summer 2019, Spring 2020, and Summer 2020. The average ratio of chlorophyll a and chlorophyll b ranged from 2.2 to 3.0 in IPC-covered trees and from 2.0 to 3.0 in trees without IPCs. A significant effect of tree cover was observed only in Spring 2020, when trees with IPCs had a larger chlorophyll a/b ratio (2.2) than trees without IPCs (2.0). There were no significant effects of the insecticide rate or the tree cover × insecticide rate interaction for any of the variables. There was a significant positive correlation between the cycle threshold values and chlorophyll contents (correlation coefficients: R = 0.5, R = 0.38, and R = 0.81 during Summer 2019, Spring 2020, and Summer 2020, respectively).

Table 1. Leaf soluble and nonsoluble carbohydrate contents.

Factors	Glucose (µg/mg)			Fructose (µg/mg)			Sucrose (µg/mg)			Starch (µg/mg)		
	Summer 2019	Spring 2020	Summer 2020	Summer 2019	Spring 2020	Summer 2020	Summer 2019	Spring 2020	Summer 2020	Summer 2019	Spring 2020	Summer 2020
Tree cover												
IPC	1.5	1.5	**1.2 b**	0.9	0.8	1.1	**16.3 b**	21.9	**21.5 b**	**21.2 b**	**23.2 b**	**12.0 b**
No IPC	1.6	1.7	**1.8 a**	0.7	0.7	1.2	**21.3 a**	18.8	**28.4 a**	**80.7 a**	**55.4 a**	**44.2 a**
P value	0.802	0.328	**0.002****	0.281	0.971	0.746	**<0.001*****	0.142	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****
Insecticide rate												
Full	1.3	1.5	1.3	0.7	0.9	0.8	19.2	22.4	22.7	47.6	42.1	19.7
Half	1.8	1.5	1.8	1	0.6	1.4	19.4	20.2	26	59.1	42.2	34.2
Zero	1.5	1.8	1.4	0.7	0.8	1.1	17.8	18.6	26.2	46.3	33.7	30.5
P value	0.34	0.306	0.124	0.348	0.239	0.273	0.516	0.332	0.065	0.557	0.6	0.101
Tree cover × insecticide rate												
IPC × full	1.2	1.4	1.1	0.8	0.9	1.1	14.6	24.4	19.4	29	28.6	10.8
IPC × half	2	1.6	1.2	1.1	0.5	1	17.4	22.4	22.4	28	25.1	13.5
IPC × zero	1.3	1.5	1.2	0.8	0.8	1.2	16.7	19	22.8	24.9	16	12
No IPC × full	1.3	1.5	1.5	0.6	0.8	0.6	23.7	20.3	26	105.9	55.6	28.6
No IPC × half	1.7	1.5	2.4	0.9	0.6	1.9	21.3	18	29.6	130.9	59.3	54.9
No IPC × zero	1.7	2.1	1.6	0.7	0.8	1.1	18.9	18.1	29.5	103.9	51.5	49
P value	0.669	0.291	0.135	0.906	0.882	0.141	0.063	0.751	0.975	0.6	0.889	0.195

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, **, *** *P* values significant at 5%, 1%, and <0.1%.

Leaf nutrients

Most of the leaf nutrient concentrations were significantly influenced by tree cover except for P, K, and Fe (Table 3). Furthermore, N, Mg, Ca, S, and Zn concentrations were significantly higher in leaves from trees covered with IPCs compared with noncovered trees. Concentrations in covered trees were 2.5% N, 28 ppm Zn, 0.49% Mg, 3.6% Ca, and 0.3% S, whereas they were 2.3% N, 22 ppm Zn, 0.42% Mg, 3.0% Ca, and 0.2% S in noncovered trees. Additionally, B, Mn, and Cu were found in significantly lower concentrations in trees with IPCs compared with trees without IPCs. Leaf concentrations of covered trees were 103 ppm B, 32 ppm Mn, and 22 ppm Cu, whereas concentrations for trees without IPCs were 111 ppm B, 41 ppm Mn, and 40 ppm Cu. The macronutrients and micronutrients were not impacted significantly by the insecticide rate and tree cover × insecticide rate interaction.

Discussion

During this study, we evaluated the effect of IPCs on different tree physiological variables and the interactions with different rates of insecticides. IPCs prevented trees from becoming infected with *C*Las, as previously reported (Gaire et al. 2022). In contrast, all trees without IPCs became infected and developed the symptoms typically associated with HLB, including chlorotic and blotchy mottled leaves and canopy die-back. Most of the tree physiological variables measured were influenced by IPCs. Leaves of trees without IPCs contained more sucrose and glucose than trees with IPCs, whereas no difference was found for the concentration of fructose. Similarly, Fan et al. (2010) found accumulations of sucrose and glucose, but not fructose, in greenhouse-grown, graft-inoculated *C*Las-infected trees. The higher sucrose and glucose levels of the trees without IPCs were likely the result of impaired photoassimilate transport caused by *C*Las infection (Albrecht and Bowman 2008; Kim et al. 2009). Sucrose is the major photosynthetic product that is transported from source leaves to sink organs, such as roots and fruits, through the phloem (Ward et al. 1997; Zimmermann and Ziegler 1975). Degeneration of phloem tissue and subsequent phloem collapse exhibited in HLB-affected trees are believed to obstruct the transport of sucrose to the sink organs, leading to its accumulation in the leaf tissue, followed by higher glucose levels after hydrolysis (Brodersen et al. 2014; Fan et al. 2010). Although hydrolysis of sucrose produces both glucose and fructose, the accumulation of glucose, but not fructose, in leaves of HLB-affected noncovered trees could be caused by the preferential use of fructose by *C*Las, as hypothesized by André et al. (2005) for *Spiroplasma citri*, which, like *C*Las, resides in the phloem.

It has long been known that the accumulation of starch is one of the most prominent characteristics of HLB-affected trees (Albrecht, and Bowman 2008; Etxeberria et al. 2009;

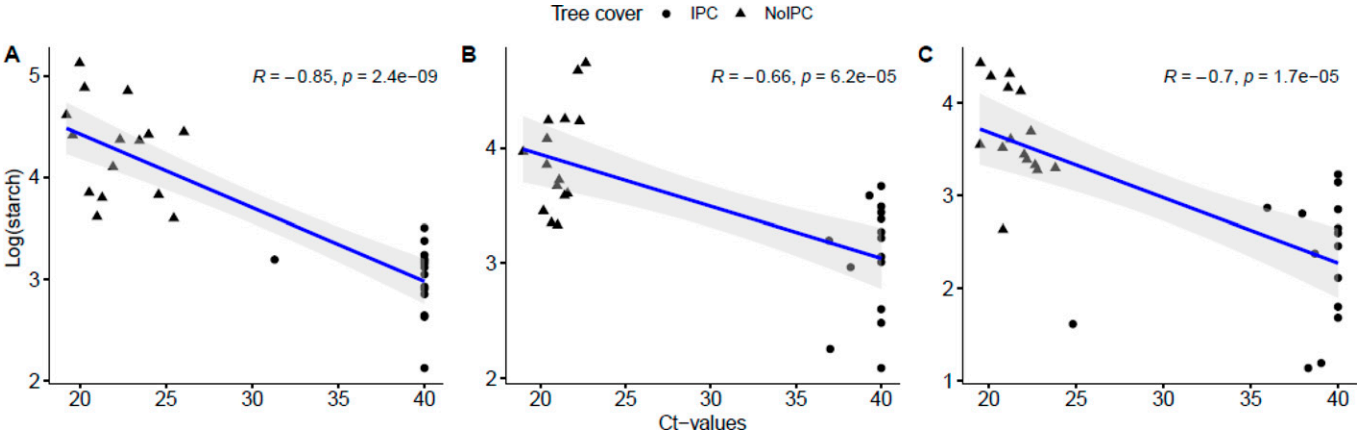

Fig. 2. Scatter plot showing correlation between leaf cycle threshold values and log-transformed starch contents measured during Summer 2019 (**A**), Spring 2020 (**B**), and Summer 2020 (**C**).

Fan et al. 2010; Kim et al. 2009; Schneider, 1968; Whitaker et al. 2014). During our study, the leaf starch content of *C*Las-infected noncovered trees was increased by more than two-fold compared with *C*Las-free trees that had been covered by IPCs. This was confirmed by the negative correlation between starch and the cycle threshold value found during our study. The increase of leaf starch in trees without IPCs could also be caused by the upregulation of starch biosynthesis enzymes and downregulation of starch degradation enzymes in response to *C*Las infection as well as downregulation of photosynthesis and chlorophyll-associated genes (Albrecht and Bowman 2008; Kim et al. 2009). Taken together, these results indicate that IPCs sustain a normal carbohydrate metabolism by preventing *C*Las infection, thereby maintaining the balance of starch and chlorophyll in the leaves.

More chlorophyll a, chlorophyll b, and total chlorophyll were found in trees that were covered with IPCs than in noncovered trees. Lower chlorophyll levels associated with excessive foliar starch accumulation induced by *C*Las infection were also found during other studies (Fan et al. 2010; Pitino et al. 2020). This supports our finding that IPCs maintain healthy levels of starch and chlorophyll. A previous study by Bondada and Syvertsen (2005) suggested a loss of the structural integrity of chloroplasts caused by starch granules and the reduction in chlorophyll concentration in N-deficient citrus leaves. A sufficient N supply is particularly important for the mobilization of starch out of the chloroplast (Ariovich and Cresswell 1983). Therefore, the combined effects of *C*Las infection, starch accumulation, and low N concentration that we observed in trees without IPCs could have contributed to the lower content in chlorophyll pigments in noncovered trees. It is generally recognized that leaf chlorophyll levels are positively correlated with the photosynthesis rate and plant productivity (Dawson et al. 2003; Gitelson et al. 2006; Gogoi and Basumatary 2018; Whittaker and Marks 1975). Therefore, the trees with IPCs are expected to be more productive because they reach maturity compared with trees without IPCs.

Plants grown under low-light intensities are generally known to have higher total chlorophyll, chlorophyll a, and chlorophyll b per unit weight of leaf and a lower chlorophyll a/b ratio (Boardman 1977). Artificially induced partial shading has also been found to increase leaf chlorophyll in different citrus species (Brand 1997; Budiarto et al. 2019; Incesu et al. 2014; Shao et al. 2014). Incesu et al. (2014) reported that in 'Lane Late' navel orange seedlings, shade net treatment induced both chlorophyll a and chlorophyll b. An increase in chlorophyll b, but not chlorophyll a, was found by Budiarto et al. (2019) in shade-grown Kafir lime. The shading effect of the IPCs likely contributed to the increased chlorophyll levels measured during our study.

Leaf macronutrient and micronutrient concentrations analyzed at the end of the study varied significantly between trees with and without IPCs, except for P, K, and Fe. Foliar N, Mg, Ca, S, and Zn concentrations of noncovered trees were significantly lower compared with those of covered trees. The lower concentration of these nutrients was likely caused by the restricted nutrient uptake and

Table 2. Leaf chlorophyll a and chlorophyll b contents.

	Chlorophyll a (μg/mg)			Chlorophyll b (μg/mg)			Total chlorophyll (μg/mg)			Chlorophyll a/b		
Factor	Summer 2019	Spring 2020	Summer 2020	Summer 2019	Spring 2020	Summer 2020	Summer 2019	Spring 2020	Summer 2020	Summer 2019	Spring 2020	Summer 2020
Tree cover												
IPC	**5.1 a**	**4.1 a**	**3.1 a**	**1.8 a**	1.9	**1.0 a**	**6.9 a**	**6.0 a**	**4.2 a**	2.8	**2.2 a**	3.0
No IPC	**4.2 b**	**3.4 b**	**2.0 b**	**1.4 b**	1.7	**0.6 b**	**5.5 b**	**5.1 b**	**2.6 b**	3.0	**2.0 b**	3.0
P value	**0.016***	**0.029***	**<0.001*****	**0.002****	0.051	**<0.001*****	**0.008****	**0.033***	**<0.001*****	0.092	**0.020***	0.607
Insecticide rate												
Full	5.2	3.5	2.4	1.8	1.7	0.8	6.9	5.2	3.2	3.0	2.1	3.0
Half	4.3	4.2	2.6	1.6	1.9	0.9	5.8	6.1	3.5	2.8	2.1	3.1
Zero	4.5	3.7	2.6	1.5	1.8	0.9	5.9	5.5	3.4	3.0	2.1	3.1
P value	0.119	0.2	0.424	0.198	0.201	0.718	0.136	0.206	0.497	0.113	0.455	0.262
Tree cover × insecticide rate												
IPC × full	5.6	3.9	3.0	2.0	1.8	1.0	7.6	5.7	4.1	2.9	2.2	2.9
IPC × half	4.8	4.8	3.4	1.8	2.1	1.1	6.6	6.9	4.5	2.7	2.3	3.0
IPC × zero	4.9	3.7	3.0	1.7	1.8	1.0	6.6	5.5	4.0	2.9	2.1	3.1
No IPC × full	4.7	3.1	1.7	1.6	1.6	0.6	6.3	4.6	2.3	3.0	1.9	3.0
No IPC × half	3.7	3.5	1.8	1.3	1.7	0.6	5.0	5.2	2.5	2.8	2.1	3.1
No IPC × zero	4.0	3.7	2.2	1.3	1.8	0.7	5.3	5.5	2.9	3.2	2.1	3.0
P value	0.947	0.253	0.175	0.921	0.312	0.158	0.940	0.266	0.167	0.873	0.336	0.688

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, **, *** *P* values significant at 5%, 1%, and <0.1%.

Table 3. Leaf nutrient concentrations in Summer (August) 2020.

Factor	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	B (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Recommended optimum[1]											
	2.5–2.7	0.12–0.16	1.20–1.70	3.0–4.9	0.30–0.49	0.2–0.4	60–120	36–100	25–100	25–100	5–16
Tree cover											
IPC	**2.5 a**	0.16	1.18	**3.6 a**	**0.49 a**	**0.3 a**	61	**103 b**	**32 b**	**28 a**	**22 b**
No IPC	**2.3 b**	0.16	1.13	**3.0 b**	**0.42 b**	**0.2 b**	62	**111 a**	**41 a**	**22 b**	**40 a**
P value	**0.003****	0.096	0.346	**<0.001*****	**<0.001*****	**<0.001*****	0.784	**0.015***	**0.019***	**<0.001*****	**<0.001*****
Insecticide rate											
Full	2.4	0.16	1.11	3.3	0.46	0.3	63	103	38	26.6	30
Half	2.3	0.16	1.21	3.4	0.46	0.3	59	107	32	24.5	27
Zero	2.4	0.16	1.16	3.3	0.44	0.3	62	110	39	22.6	35
P value	0.513	0.483	0.356	0.473	0.102	0.091	0.455	0.221	0.217	0.101	0.081
Tree cover × insecticide rate											
P value	0.972	0.195	0.455	0.417	0.258	0.205	0.383	0.724	0.213	0.278	0.33

[1] Based on the work by Zekri and Obreza (2019) and Morgan et al. (2020).

Different letters within columns indicate significant differences according to Tukey's honestly significant difference test. *, **, *** *P* values significant at 5%, 1%, and <0.1%.

transport, which have been reported for HLB-affected citrus trees (Nwugo et al. 2013; Spann and Schumann 2009; Wang and Trivedi 2013). Based on current guidelines for citrus (Morgan et al. 2020), foliar N and Zn were deficient in trees without IPCs during our study. As discussed, N deficiency could have exacerbated the low chlorophyll content per unit area and high starch content in chloroplast, as reported by other studies (Ariovich and Cresswell 1983; Bondada and Syvertsen 2003).

In contrast to N, Mg, Ca, S, and Zn, the micronutrients B, Mn, and Cu were found in significantly lower concentrations in the leaves of trees that had been covered with IPCs than in noncovered trees. This is inconsistent with the results of a previous study during which those micronutrients were significantly reduced in HLB-affected trees (Spann and Schumann 2009). The higher concentration of Cu in the leaves of noncovered trees was likely caused by the frequent applications of this metal to control citrus canker (Behlau et al. 2008). IPCs might have prevented the foliar Cu spray to reach the leaves of covered trees, resulting in lower foliar concentrations. High Cu concentrations were also found in the soil at our trial site (Supplemental Table 3). Although leaf B and Mn concentrations were lower under IPCs, both micronutrients were in the greater than optimal and optimal range, respectively. The reason for the lower B and Mn concentrations in the trees with IPCs is unclear and requires further investigation.

Although no significant differences in foliar K concentrations were found between trees with and without IPCs, foliar K concentrations were in the less than optimal range, irrespective of tree cover. The relatively low K concentrations in the leaves might be attributable to the low soil K concentration that was measured at the trial site (Supplemental Table 3). Most of the other foliar macronutrient and micronutrient concentrations were either within the optimal range or higher based on the current guidelines for citrus (Morgan et al. 2020; Zekri and Obreza 2019). Proper nutrient management during the trial location is likely responsible for the adequate concentration of these nutrients in the leaves.

Conclusion

The HLB-induced accumulations of starch, sucrose, and glucose in the leaves were prevented using IPCs, whereas no significant influence was reported for fructose. IPCs maintained both chlorophyll a and chlorophyll b levels in leaves and prevented an HLB-induced deficiency of foliar N and Zn while maintaining a higher concentration of many of the other nutrients. Overall, the results of this study indicate that IPCs are effective not only for excluding psyllids and preventing *C*Las infection but also for maintaining the physiological health of young citrus trees. The use of IPCs to protect young citrus trees from HLB in a disease-endemic environment, such as in Florida, is highly recommended as part of an integrated pest management program.

References Cited

Achor DS, Etxeberria E, Wang N, Folimonova SY, Chung KR, Albrigo LG. 2010. Sequence of anatomical symptom observations in citrus affected with huanglongbing disease. Plant Pathol J. 9:56–64. https://doi.org/10.3923/ppj.2010.56.64.

Albrecht UKD, Bowman KD. 2008. Gene expression in *Citrus sinensis* (L.) Osbeck following infection with the bacterial pathogen *Candidatus* Liberibacter asiaticus causing huanglongbing in Florida. Plant Sci. 175(3):291–306. https://doi.org/10.1016/j.plantsci.2008.05.001.

Alferez F, Albrecht U, Gaire S, Batuman O, Qureshi J, Zekri M. 2021. Individual protective covers (IPCs) for young tree protection from the HLB vector, the Asian citrus psyllid. UF/IFAS EDIS HS1425.

André A, Maucourt M, Moing A, Rolin D, Renaudin J. 2005. Sugar import and phytopathogenicity of *Spiroplasma citri*: Glucose and fructose play distinct roles. Mol Plant Microbe Interact. 18(1):33–42. https://doi.org/10.1094/MPMI-18-0033.

Archer L, Kunwar S, Alferez F, Batuman O, Albrecht U. 2022a. Trunk injection of oxytetracycline for huanglongbing management in mature grapefruit and sweet orange trees. Phytopathology. https://doi.org/10.1094/PHYTO-09-22-0330-R (In press).

Archer L, Qureshi J, Albrecht U. 2022b. Efficacy of trunk injected imidacloprid and oxytetracycline in managing huanglongbing and Asian citrus psyllid in infected sweet orange (*Citrus sinensis*) trees. Agriculture. 12(10):1592. https://doi.org/10.3390/agriculture12101592.

Ariovich D, Cresswell CF. 1983. The effect of nitrogen and phosphorus on starch accumulation and net photosynthesis in two variants of *Panicum maximum* Jacq. Plant Cell Environ. 6(8):657–664. https://doi.org/10.1111/1365-3040.ep11589234.

Aritua V, Achor D, Gmitter FG, Albrigo G, Wang N. 2013. Transcriptional and microscopic analyses of citrus stem and root responses to *Candidatus* Liberibacter asiaticus infection. PLoS One. 8(9):e73742. https://doi.org/10.1371/journal.pone.0073742.

Behlau F, Belasque Jr J, Bergamin Filho A, Graham JH, Leite Jr RP, Gottwald TR. 2008. Copper sprays and windbreaks for control of citrus canker on young orange trees in southern Brazil. Crop Prot. 27(3–5):807–813. https://doi.org/10.1016/j.cropro.2007.11.008.

Berlinger MJ, Taylor RAJ, Lebiush-Mordechi S, Shalhevet S, Spharim I. 2002. Efficiency of insect exclusion screens for preventing whitefly transmission of tomato yellow leaf curl virus of tomatoes in Israel. Bull Entomol Res. 92(5):367–373. https://doi.org/10.1079/BER2002180.

Boardman NT. 1977. Comparative photosynthesis of sun and shade plants. Annu Rev Plant Physiol. 28(1):355–377. https://doi.org/10.1146/annurev.pp.28.060177.002035.

Boina DR, Bloomquist JR. 2015. Chemical control of Asian citrus psyllid and of huanglongbing disease in citrus. Pest Manag Sci. 71(6):808–823. https://doi.org/10.1002/ps.3957.

Bondada BR, Syvertsen JP. 2003. Leaf chlorophyll, net gas exchange and chloroplast ultrastructure in citrus leaves of different nitrogen status. Tree Physiol. 23(8):553–559. https://doi.org/10.1093/treephys/23.8.553.

Bondada BR, Syvertsen JP. 2005. Concurrent changes in net CO_2 assimilation and chloroplast ultrastructure in nitrogen deficient citrus leaves. Environ Exp Bot. 54(1):41–48. https://doi.org/10.1016/j.envexpbot.2004.05.005.

Bove JM. 2006. Huanglongbing: A destructive newly emerging, century-old disease of citrus. Plant Pathol. 88(1):7–37. https://www.jstor.org/stable/41998278?seq=1#metadata_info_tab_contents.

Bowman KD, Albrecht U. 2020. Rootstock influences on health and growth following *Candidatus* Liberibacter asiaticus infection in young sweet orange trees. Agron J. 10(12):1907. https://doi.org/10.3390/agronomy10121907.

Bowman KD, McCollum G, Albrecht U. 2016. Performance of 'Valencia' orange [*Citrus sinensis* (L.) Osbeck] on 17 rootstocks in a trial

severely affected by huanglongbing. Scientia Hortic. 201:355–361. https://doi.org/10.1016/j.scienta.2016.01.019.

Brand MH. 1997. Shade influences plant growth, leaf color, and chlorophyll content of *Kalmia latifolia* L. cultivars. HortScience. 32(2):206–208. https://doi.org/10.21273/HORTSCI.32.2.206.

Brodersen C, Narciso C, Reed M, Etxeberria E. 2014. Phloem production in Huanglongbing-affected citrus trees. HortScience. 49(1):59–64. https://doi.org/10.21273/HORTSCI.49.1.59.

Budiarto R, Poerwanto R, Santosa E, Efendi D, Agusta A. 2019. Agronomical and physiological characters of Kaffir lime (*Citrus hystrix* DC) seedling under artificial shading and pruning. Emir J Food Agric. 31:222–230. https://doi.org/10.9755/ejfa.2019.v31.i3.1920.

Chung KR, Brlansky RH. 2005. Citrus diseases exotic to Florida: Huanglongbing (citrus greening). EDIS, 2005(7). Citrus Diseases Exotic to Florida: Huanglongbing (Citrus Greening) EDIS (flvc.org).

Dala-Paula BM, Plotto A, Bai J, Manthey JA, Baldwin EA, Ferrarezi RS, Gloria M. 2019. Effect of huanglongbing or greening disease on orange juice quality, a review. Front. Plant Sci. 9:1976. https://doi.org/10.3389/fpls.2018.01976.

Dawson TP, North PJR, Plummer SE, Curran PJ. 2003. Forest ecosystem chlorophyll content: Implications for remotely sensed estimates of net primary productivity. Int J Remote Sens. 24(3):611–617. https://doi.org/10.1080/01431160304984.

de Carvalho DU, Boakye D, Gast T, Pereira Leite Jr R, Alferez F. 2021. Determining seed viability during fruit maturation to improve seed production and availability of new citrus rootstocks. Front Plant Sci. 12:777078. https://doi.org/10.3389/fpls.2021.777078.

Deng H, Achor D, Exteberria E, Yu Q, Du D, Stanton D, Liang G, Gmitter FG Jr. 2019. Phloem regeneration is a mechanism for huanglongbing-tolerance of 'Bearss' lemon and 'LB8-9' Sugar Belle® mandarin. Front. Plant Sci. 10:277. https://doi.org/10.3389/fpls.2019.00277.

Ebert TA, Schumann AW, Waldo L, Stanton D, Rolshausen P. 2021. A close-up look at screens for excluding Asian citrus psyllids: SS702/SL489, 6/2021. EDIS.

Etxeberria E, Gonzalez P, Achor D, Albrigo G. 2009. Anatomical distribution of abnormally high levels of starch in HLB-affected Valencia orange trees. Physiol Mol Plant Pathol. 74(1):76–83. https://doi.org/10.1016/j.pmpp.2009.09.004.

Fan J, Chen C, Brlansky RH, Gmitter FG Jr, Li ZG. 2010. Changes in carbohydrate metabolism in *Citrus sinensis* infected with *Candidatus* Liberibacter asiaticus. Plant Pathol. 59(6):1037–1043. https://doi.org/10.1111/j.1365-3059.2010.02328.x.

Ferrarezi RS, Qureshi JA, Wright AL, Ritenour MA, Macan NPF. 2019. Citrus production under screen as a strategy to protect grapefruit trees from huanglongbing disease. Front. Plant Sci. 10:1598. https://doi.org/10.3389/fpls.2019.01598.

Gaire S, Albrecht U, Batuman O, Qureshi J, Zekri M, Alferez F. 2022. Individual protective covers (IPCs) to prevent Asian citrus psyllid and *Candidatus* Liberibacter asiaticus from establishing in newly planted citrus trees. Crop Prot. 152:105862. https://doi.org/10.1016/j.cropro.2021.105862.

Gitelson AA, Viña A, Verma SB, Rundquist DC, Arkebauer TJ, Keydan G, Leavitt B, Ciganda V, Burba GG, Suyker AE. 2006. Relationship between gross primary production and chlorophyll content in crops: Implications for the synoptic monitoring of vegetation productivity. J Geophys Res Atmos. 111(D8). https://doi.org/10.1029/2005JD006017.

Gogoi M, Basumatary M. 2018. Estimation of the chlorophyll concentration in seven Citrus species of Kokrajhar district, BTAD, Assam, India. Trop. Plant Research. 5(1):83–87.

Gomez L, Bancel D, Rubio E, Vercambre G. 2007. The microplate reader: An efficient tool for the separate enzymatic analysis of sugars in plant tissues—Validation of a micro-method. J Sci Food Agr. 87(10):1893–1905. https://doi.org/10.1002/jsfa.2924.

Gottwald TR. 2010. Current epidemiological understanding of citrus huanglongbing. Annu Rev Phytopathol. 48:119–139. https://doi.org/10.1146/annurev-phyto-073009-114418.

Graham J, Gottwald T, Setamou M. 2020. Status of huanglongbing (HLB) outbreaks in Florida, California, and Texas. Trop Plant Pathol. https://doi.org/10.1007/s40858-020-00335-y.

Haijun L, Cohen S, Lemcoff JH, Israeli Y, Tanny J. 2015. Sap flow, canopy conductance and microclimate in a banana screenhouse. Agr For Meteorol. 201:165–175. https://doi.org/10.1016/j.agrformet.2014.11.009.

Halbert SE. 2005. The discovery of huanglongbing in Florida, p 50. Proceedings of the 2nd International Citrus Canker and Huanglongbing Research Workshop 2005, Orlando FL, USA.

Halbert SE, Manjunath KL. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: A literature review and assessment of risk in Florida. Flor Entomol. 87:330–353. https://doi.org/10.1653/0015-4040(2004)087[0330:ACPSPA]2.0.CO;2.

Hall DG, Albrecht U, Bowman KD. 2016. Transmission rates of *Ca. Liberibacter* Asiaticus by Asian citrus psyllid are enhanced by the presence and developmental stage of citrus flush. J Econ Entomol. 109:558–563. https://doi.org/10.1093/jee/tow009.

Havlin JL, Soltanpour PN. 1980. A nitric acid plant tissue digest method for use with inductively coupled plasma spectrometry. Commun Soil Sci Plant Anal. 11:969–980. https://doi.org/10.1080/00103628009367096.

Huang CYL, Schulte EE. 1985. Digestion of plant tissue for analysis by ICP emission spectroscopy. Commun Soil Sci Plant Anal. 16(9):1–10. https://doi.org/10.1080/00103628509367657.

Incesu M, Yeşiloğlu T, Çimen B, Yõlmaz B. 2014. Effects of nursery shading on plant growth, chlorophyll content and PSII in 'Lane Late' navel orange seedlings. In: XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014). 1130:301–306. https://doi.org/10.17660/ActaHortic.2016.1130.44.

Johnson EG, Wu J, Bright DB, Graham JH. 2014. Association of *Candidatus* Liberibacter asiaticus root infection, but not phloem plugging with root loss on huanglongbing-affected trees prior to appearance of foliar symptoms. Plant Pathol. 63(2):290–298. https://doi.org/10.1111/ppa.12109.

Kim JS, Sagaram US, Burns JK, Li JL, Wang N. 2009. Response of sweet orange (*Citrus sinensis*) to *Candidatus* Liberibacter asiaticus infection: Microscopy and microarray analyses. Phytopathology. 99(1):50–57. https://doi.org/10.1094/PHYTO-99-1-0050.

Koh EJ, Zhou L, Williams DS, Park J, Ding N, Duan YP, Kang BH. 2012. Callose deposition in the phloem plasmodesmata and inhibition of phloem transport in citrus leaves infected with *Candidatus* Liberibacter asiaticus. Protoplasma. 249(3):687–697. https://doi.org/10.1007/s00709-011-0312-3.

Kumar N, Kiran F, Etxeberria E. 2018. Huanglongbing-induced anatomical changes in citrus fibrous root orders. HortScience. 53(6):829–837. https://doi.org/10.21273/HORTSCI12390-17.

Kunwar S, Grosser J, Gmitter FG, Castle WS, Albrecht U. 2021. Field performance of 'Hamlin' orange trees grown on various rootstocks in huanglongbing-endemic conditions. HortScience. 56:244–253. https://doi.org/10.21273/HORTSCI15550-20.

Kunwar S, Meyering B, Grosser J, Gmitter FG Jr, Castle WS, Albrecht U. 2023. Field performance of 'Valencia' orange trees on diploid and tetraploid rootstocks in different huanglongbing-endemic growing environments. Scientia Hortic. 309:111635. https://doi.org/10.1016/j.scienta.2022.111635.

Mahmood A, Hu Y, Tanny J, Asante EA. 2018. Effects of shading and insect-proof screens on crop microclimate and production: A review of recent advances. Scientia Hortic. 241:241–251. https://doi.org/10.1016/j.scienta.2018.06.078.

Mattos D Jr, Kadyampakeni DM, da Silva JR, Vashisth T, Boaretto RM. 2020. Reciprocal effects of huanglongbing infection and nutritional status of citrus trees: A review. Trop Plant Pathol. 45:586–596. https://doi.org/10.1007/s40858-020-00389-y.

Medina CL, Saccini VAV, Dos Santos DMM, Machado RS, Bataglia OC, Furlani PR. 2014. seasonal concentration of macro and micronutrients in different vegetative organs of Valencia oranges tree affected by HLB. J Citrus Pathol. 1(1):242. https://escholarship.org/uc/item/03h187mc.

Morgan KT, Kadyampakeni D, Zekri M, Schumann AW, Vashisth T, Obreza TA. 2020. 2020–2021 Florida citrus production guide: Nutrition management for citrus trees. EDIS. CMG13 (CG091):2020–2021. Florida Citrus Production Guide: Nutrition Management for Citrus Trees (ufl.edu).

Mylavarapu R, Harris W, Hochmuth G. 2016. Agricultural soils of Florida. University of Florida IFAS Extension publication SL441. https://edis.ifas.ufl.edu/ss655.

Nayek S, Choudhary IH, Jaishee N, Roy S. 2014. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. Research J Chemical Sciences. 4:63–69. https://www.academia.edu/download/40169896/SN_Chlorophylls_2014.pdf.

Nwugo CC, Lin H, Duan Y, Civerolo EL. 2013. The effect of *Candidatus* Liberibacter asiaticus infection on the proteomic profiles and nutritional status of pre-symptomatic and symptomatic grapefruit (*Citrus paradisi*) plants. BMC Plant Biol. 13(1):1–24. https://doi.org/10.1186/1471-2229-13-59.

Pitino M, Sturgeon K, Dorado C, Cano LM, Manthey JA, Shatters RG Jr, Rossi L. 2020. *Quercus* leaf extracts display curative effects against *Candidatus* Liberibacter asiaticus that restore leaf physiological parameters in HLB-affected citrus trees. Plant Physiol Biochem. 148:70–79. https://doi.org/10.1016/j.plaphy.2020.01.013.

Pokhrel S, Meyering B, Bowman KD, Albrecht U. 2020. Horticultural attributes and root architectures of field-grown 'Valencia' trees grafted on different rootstocks propagated by seed, cuttings, and tissue culture. HortScience. 1:1–10. https://doi.org/10.21273/HORTSCI15507-20.

Polston JE, Lapidot M. 2007. Management of tomato yellow leaf curl virus: US and Israel perspectives, p 251–262. In: Czosnek H (ed). Tomato yellow leaf curl virus disease. Springer, Dordrecht, the Netherlands. https://doi.org/10.1007/978-1-4020-4769-5_15.

Rodrigues JD, Moreira AS, Stuchi ES, Bassanezi RB, Laranjeira FF, Girardi EA. 2020. Huanglongbing incidence, canopy volume, and sprouting dynamics of 'Valencia' sweet orange grafted onto 16 rootstocks. Trop Plant Pathol. 45:611–619. https://doi.org/10.1007/s40858-020-00385-2.

Rogers M. Young tree care. 2014. UF/IFAS Extension. May 2016. http://www.crec.ifas.ufl.edu/extension/chmas/PDF/Young_Tree_Care.pdf.

Rogers ME, Stansly PA, Stelinski LL. 2012. Florida citrus pest management guide: Asian citrus psyllid and Citrus leafminer. Entomol. Nematol. Dept., Fla. Coop. 00003029.pdf (ufl.edu).

Schaffer AA, Liu KC, Goldschmidt EE, Boyer CD, Goren R. 1986. Citrus leaf chlorosis induced by sink removal: Starch, nitrogen, and chloroplast ultrastructure. J Plant Physiol. 124(1–2): 111–121. https://doi.org/10.1016/S0176-1617(86)80183-3.

Schneider H. 1968. Anatomy of greening-diseased sweet orange shoots. Phytopathology. 58:1155–1160. schneider68.pdf (ufl.edu).

Schumann AW, Singerman A, Wright A, Ferrarezi RS, Qureshi J, Alferez F. 2021. 2021–2022 Florida citrus production guide: Citrus under protective screen (CUPS) production systems. EDIS HS1304. https://edis.ifas.ufl.edu/publication/HS1304.

Shahbaz E, Ali M, Shafiq M, Atiq M, Hussain M, Balal RM, Sarkhosh A, Alferez F, Sadiq S, Shahid MA. 2023. Citrus canker pathogen, its mechanism of infection, eradication, and impacts. Plants. 12:123. https://doi.org/10.3390/plants12010123.

Shao Q, Wang H, Guo H, Zhou A, Huang Y, Sun Y, Li M. 2014. Effects of shade treatments on photosynthetic characteristics, chloroplast ultrastructure, and physiology of *Anoectochilus roxburghii*. PLoS One. 9(2):e85996. https://doi.org/10.1371/journal.pone.0085996.

Singh B, Kumar M, Singh V. 2006. Nylon mesh screens reduce incidence of leaf curl virus and improve yield in sweet pepper. J Veg Sci. 12(1):65–70. https://doi.org/10.1300/J484v12n01_06.

Spann TM, Schumann AW, 2009. The role of plant nutrients in disease development with emphasis on citrus and huanglongbing. Proc Fla State Hort Soc. 122:169–171. FSHS09.indb (ufl.edu).

Stansly PA, Arevalo HA, Qureshi JA, Jones MM, Hendricks K, Roberts PD, Roka FM. 2014. Vector control and foliar nutrition to maintain economic sustainability of bearing citrus in Florida groves affected by huanglongbing. Pest Manag Sci. 70(3):415–426. https://doi.org/10.1002/ps.3577.

Sweeney RA. 1989. Generic combustion method for determination of crude protein in feeds: Collaborative study. J Assn Offic Anal Chem. 72(5):1–10. https://doi.org/10.1093/jaoac/72.5.770.

US Department of Agriculture, National Agricultural Statistics Service. 2019. Florida Citrus Statistics. https://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/Citrus_Statistics/2018-19/fcs1819.pdf.

Vincent C, Guha A, Killiny N, Diepenbrock L. 2021. Understory environment promotes photosynthetic efficiency and mitigates severity and function of an introduced, vectored pathosystem: A study of a feral citrus population in central Florida. Trees (Berl). 35(5):1711–1725. https://doi.org/10.1007/s00468-021-02147-2.

Wang N, Trivedi P. 2013. Citrus huanglongbing: A newly relevant disease presents unprecedented challenges. Phytopathology. 103(7):652–665. https://doi.org/10.1094/PHYTO-12-12-0331-RVW.

Ward JM, Kühn C, Tegeder M, Frommer WB. 1997. Sucrose transport in higher plants. Int Rev Cytol. 178:41–71. https://doi.org/10.1016/S0074-7696(08)62135-X.

Whitaker DC, Giurcanu MC, Young LJ, Gonzalez P, Etxeberria E, Roberts P, Katherine H, Roman F. 2014. Starch content of citrus leaves permits diagnosis of huanglongbing in the warm season but not cool season. HortScience. 49(6): 757–762. https://doi.org/10.21273/HORTSCI.49.6.757.

Whittaker RH, Marks PL. 1975. Methods of assessing terrestrial productivity, p 55–118. In: Lieth H, Whittaker RH (eds). Primary productivity of the biosphere. Ecological studies, vol 14. Springer, Berlin, Heidelberg, Germany. https://doi.org/10.1007/978-3-642-80913-2_4.

Zekri M, Obreza T. 2019. Calcium (Ca) and sulfur (S) for citrus trees: SL382/SS584, 7/2013 HISTORICAL FACT SHEETS. https://doi.org/10.32473/edis-ss584-2013.

Zimmermann MH, Ziegler H. 1975. List of sugars and sugar alcohols in sieve-tube exudates (Appendix 3), p 480–483. In: Zimmerman MH, Milburn JA (eds). Transport in plants 1. Phloem transport. Encyclopedia of plant physiology series (Vol. 1). Springer-Verlag, Heidelberg, Germany.