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Research Article

Interaction between *Phytophthora nicotianae* and *Candidatus* Liberibacter asiaticus damage to citrus fibrous roots

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Abstract

Huanglongbing (HLB) is associated with the phloem-limited bacterium, *Candidatus* Liberibacter asiaticus (Las). *Phytophthora nicotianae* (*P.n.*) causes root rot of citrus, which reduces water and nutrient uptake by citrus fibrous roots. The discovery that Las damage to fibrous roots occurs before tree canopy symptoms develop led to the prediction that Las root infection directly damages roots and may interact with soil-borne pathogens to cause further damage. Hence, comparison of root damage by Las and *P.n.* alone or in combination was carried out on seedlings of Cleopatra mandarin (*Citrus reticulata* Blanco) rootstock to evaluate the possible interaction of Las and *P.n.* and their relative contribution to fibrous root loss. The results demonstrated that i) roots of seedlings have a similar level of damage when inoculated with Las or *P.n.*, and co-inoculation causes comparable damage as each pathogen alone; and ii) Las infection increases and decreases *P.n.* infection incidence overtime without following a clear progression.

Keywords: Citrus fibrous root, carbohydrate, interaction

Introduction

Huanglongbing (HLB), also known as citrus greening in Africa or likubin in Taiwan (Ann et al. 2004; da Graca 1991), is considered the most devastating citrus disease known (Bassanezi et al. 2011; Bové 2006; Gottwald 2010). HLB in Florida is associated with the phloem-limited bacterium, *Candidatus* Liberibacter asiaticus (Las) (Jagoueix et al. 1994), which is transmitted from tree to tree by the psyllid vector *Diaphorina citri*, or by grafting infected tissue from other hosts of the pathogen (Garnier and Bové 1983; Halbert and Manjunath 2004).

Symptoms of HLB on leaves, fruits, and in phloem have been widely reported in previous studies (Achor et al. 2010; Bassanezi et al. 2009; Etxeberria et al. 2009; Schneider 1968). Root loss of trees with HLB was believed to be secondary damage after carbohydrate transport is disrupted in canopy phloem (Etxeberria et al. 2009; da Graca 1991; Kim et al. 2009; Koh et al. 2012). However, rapid yield loss of sweet orange trees at a low level of canopy symptom expression was observed in Brazil (Bassanezi et al. 2011). This finding indicates that undetected damage occurs before canopy symptoms develop (Graham et al. 2013). Johnson et al. (2014) investigated roots as a site for Las multiplication at an early stage of the infection process by tracking movement of Las in graft-inoculated greenhouse trees and symptomless orchard trees. Root infection and loss detected before phloem was blocked and canopy symptoms developed led to the prediction that Las root infection directly damages roots and may interact with soil-borne pathogens to cause further damage (Graham et al. 2013; Johnson et al. 2014). Meanwhile, unprecedented increases in the population of soil-borne *Phytophthora nicotianae* Breda de Haan (*P.n.*) in citrus orchards were documented as HLB spread throughout Florida (Graham et al. 2011). These findings led to the investigation of fibrous root loss and the interaction between Las and *P.n.* at the early stage of HLB development.

P.n. damages fibrous roots, which reduces water and nutrient uptake and depletes carbohydrate reserves in roots (Graham 1995). Understanding the interaction of *Phytophthora* spp. with other root pathogens and pests is important for developing the most effective management of fibrous root health (Davis and Menge 1981; Graham and Feichtenberger 2015). The availability of sugars in the host is important for synthesis of defense compounds for expression of host tolerance to *Phytophthora* spp. (Jönsson 2006).

Phytophthora spp. have been proposed to contribute to pre-symptomatic root loss on trees with HLB (Graham et al. 2013; Johnson et al. 2014). The interaction between Las and *P.n.* was first recognized by Ann et al. (2004) who evaluated, in the greenhouse, the effect on tree

growth 6 months after inoculation with each pathogen individually or together. Ann et al. (2004) demonstrated that 14 days after graft inoculation, Las increased *P.n.* infection incidence. Additionally, reduction in height was greater for seedlings infected with Las and *P.n.* than with Las alone on rootstock sour orange (*Citrus aurantium* L.) and pummelo (*Citrus grandis* (L.) Osbeck) (Ann et al. 2004). The relative contribution of each pathogen and the combination of Las and *P.n.* to fibrous root damage was not investigated (Ann et al. 2004).

The objective of this study was to evaluate Las and *P.n.* damage on citrus fibrous roots in greenhouse trials with small seedlings of the *P.n.* susceptible citrus rootstock, Cleopatra mandarin (*Citrus reticulata* Blanco).

Materials and Methods

Experimental design and inoculation with Candidatus Liberibacter asiaticus

Cleopatra mandarin seeds, obtained from Rucks Citrus Nursery, Frostproof, FL, were sown in Metro Mix 500 (Hummert International) in 120 cm^3 containers in the greenhouse. Seedlings were fertilized with 20 ml PETERS Pro 20-10-20 (Scotts-Sierra Horticultural Products Company, Marysville, OH) and 6 g Harrell's 18-5-10 (Harrell's) every other week and every 6 months, respectively, and watered 3 times per week. Four hundred grams of PETERS Pro 20-10-20 (N-P-K) was dissolved in 19 liters water and further diluted in 1:100 before application to seedlings. One hundred and twenty 6-month-old seedlings were graft inoculated with budwood from Las-infected trees. The budwood used for inoculation was from greenhouse-grown citrus cultivar 'Madam Vinous' sweet orange (*C. sinensis* (L.) Osbeck) trees infected with Las isolate FC6 as previously described (Folimonova et al. 2009). The bud was inserted into the seedling stem 12 to 17 cm above the soil line. Six months after inoculation, 60 asymptomatic Las-infected seedlings (PCR confirmed; see methods below) from 120 Las-inoculated seedlings, and 60 non-inoculated seedlings were selected for uniform size (stem height: 70 cm, stem diameter: 1 cm), and transferred into autoclaved Candler fine sand in 120 cm^3 containers for 4 treatments: *P.n.* +/- and Las +/- (10 replicates for each treatment) and 3 harvest times. The experiment was repeated twice, beginning in April 8, 2013 and May 13, 2014.

Inoculation with Phytophthora nicotianae

P.n. (Pn 198) isolated from declining Cleopatra mandarin seedlings at the University of Florida Citrus Research and Education Center, Lake Alfred, Florida, was maintained on clarified V8 juice agar. Chlamydospores were produced by the method of Tsao (1971) for inoculation. Chlamydospores harvested from liquid culture were blended, added to Candler fine sand soil and mixed manually for inoculum preparation. To assess propagule density, soil inoculum was diluted 1:10 in 0.25% water agar, spread on pimaricin-ampicillin-rifampcin-pentachloronitrobenzene-hymexazol (PARPH) semi-selective agar medium (Graham 1995), and incubated at room temperature in dark for 2 days. An inoculum density of 20 propagules per cm^3 of soil was obtained by mixing the inoculum concentrate with autoclaved Candler fine sand. The main structural root of the seedling was pruned to uniform length (10cm). In the remaining root system about half of the fibrous roots were removed uniformly from crown to bottom to allow enough space for root growth. Thirty Las-infected and 30 non-inoculated seedlings were transplanted into soil infested with *P.n.* and 30 Las-infected and 30 non-inoculated seedlings were transplanted into autoclaved Candler fine sand. Seedlings inoculated and non-inoculated with *P.n.* were located on adjacent benches in the greenhouse with the same light and temperature conditions (27 ^oC to 32 ^oC). Seedlings were watered 3 times per week and fertilized with Jack's Pro 20-20-20 every other week.

Assessment of Phytophthora nicotianae root infection incidence and fibrous root biomass

At 5, 8 and 11 weeks post inoculation (wpi) with *P.n.*, seedlings were watered to field capacity and harvested 24 h after irrigation. At harvest, seedlings from each treatment were lifted out of the containers, washed free of soil and 20 fibrous root tips (1 cm long) were arbitrarily selected from *P.n.*-inoculated and non-inoculated seedlings and plated on PARPH medium. Plates were incubated in dark for 3 to 4 days, and the percentage of *P.n.* positive tips was recorded as infection incidence (%). Fibrous roots (diameter ≤ 2mm) were separated, dried (70 °C for 48 h) and weighed.

Candidatus Liberibacter asiaticus detection

To detect Las in seedlings, 25 mg of fresh roots and 2 leaves were collected arbitrarily. Leaf midribs were chopped into square segments, placed in 2 ml screw cap tubes with one 5 mm stainless steel bead (Qiagen). The tubes were stored in a -80 °C freezer for at least 2 h and after removal from the freezer immediately ground twice at 30 revolutions per second in a Tissuelyzer II (Qiagen). Ground midrib tissue was then processed for DNA extraction using the DNeasy Plant Mini kit (Qiagen) according to the manufacturer's instructions, except that DNA was eluted twice with 50 µl AE buffer instead of twice with 100 µl (Johnson et al. 2014). Randomly selected fibrous roots were cut and ground as described above for the midrib tissue. Root DNA was extracted using the MoBio PowerSoil DNA Isolation kit (Mo Bio Laboratories) as previously described (Johnson et al. 2014). Purified DNA solution was stored at -20 °C for later analysis.

Quantitative polymerase chain reactions (qPCR) were carried out using primers and probes as previously described (Wang et al. 2006) on an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems). The master mix included Qiagen Hot Star Taq, ROX passive reference dye (Bio-Rad), primers, probe and buffer in a concentration ratio as described by the

manufacturer's protocol. Four microliters of template DNA and 16 µl master mix were loaded into each well of a 96-well micro-plate. Each sample was replicated. Samples were run in triplicate if significant variation was detected between replicates in the initial assay. The standard curve included target fragment concentration of $1x10^1$ to $1x10^6$ copies from target-containing plasmid pLBA1 (Trivedi et al. 2009).

Detection of canopy symptom development

Appearance of HLB symptoms such as blotchy mottle on leaves, yellow shoots, small leaves, and yellow vein was evaluated.

Quantification of starch and sucrose in fibrous roots

A 25 mg subsample of dried fibrous root of each seedling was randomly selected for starch analysis, and processed as previously described (Rosales and Burns 2011) with modifications. Fibrous roots were cut and ground in 2 ml tubes as described above for DNA extraction. A 900 µl aliquot of DI water was mixed with the root powder. The suspension of ground roots was vortexed and boiled for 10 min. Samples were centrifuged for 2 min at 2500 x g. A 300 µl aliquot of supernatant was stored at -20 °C for sucrose assay. Another 300 µl aliquot of supernatant was transferred into a 2 ml tube and mixed with 900 µL of ethanol. The mixture was vortexed for 5 s and centrifuged for 10 min at 10,000 x g. The supernatant was discarded and the pellet was suspended in 1.0 ml DI water by vortexing for 4 min. A 50 µl aliquot of KI:I_2 solution (120 mM: 7.8 mM) was added, vortexed for 5 s and centrifuged for 2 min at 10,000 x g. Rice starch (Sigma-Aldrich) was used as the standard for starch assay with a concentration range from 0 to 1.0 mg per ml. The mixture was transferred into a 96-well micro-plate and the absorbance at 594 nm was measured on a Benchmark Plus Microplate Spectrophotometer System (BIO-RAD, Philadelphia, PA). Sample starch concentration was calculated from absorbance reading according to the standard curve. A 2.5 µl aliquot of the stored supernatant from each sample was processed according to the manufacturer's protocol for the Glucose and Sucrose Colorimetric/Fluorometric Assay (Sigma-Aldrich). The absorbance at 570 nm was carried out on a Benchmark Plus Microplate Spectrophotometer System. Sucrose concentrations in fibrous roots were calculated from absorbance reading according to the standard curve, provided by the manufacturer, and expressed as mg sucrose per g root dry weight.

Statistical analysis

Fibrous root biomass, starch and sucrose were analyzed by two-way ANOVA, and *P.n.* infection incidence was analyzed by one-way ANOVA using PROC GLM (SAS v. 9.4). The significance level was set at $P \leq 0.05$. The relationship between total sugar content and *P.n.* infection incidence was analyzed by simple linear regression using PROC REG (SAS v. 9.4).

Results

Detection of Las population and canopy symptom development

To confirm successful Las inoculation, seedlings with pLBA2 concentration higher than $2x10^2$ copies in PCR template in either roots or leaves were considered Las positive (Trivedi et al. 2009), and selected for treatments. Las was detected in nearly 50% of the seedlings with populations ranging from 183 to $1x10^6$ copies/mg in fresh root at 3 harvests in the 2013 trial and 200 to $4x10^7$ in the 2014 trial. However, no significant difference in average Las concentration between 2 trials was detected due to the high variance. No apparent foliar symptoms were observed. Both Las-infected seedlings and non-inoculated controls displayed slight yellow vein. However, average ratio of yellow vein leaves/total leaves was comparable between Las-infected seedlings and non-inoculated controls.

Effect of Las on P.n. infection incidence

Las infection of seedlings significantly increased *P.n.* infection incidence compared to Las (-) seedlings only at 11 wpi in the 2013 trial (Fig. 1a) and only at 5 wpi in the 2014 trial (Fig. 1b). In 2013, *P.n.* infection incidence was lower for Las (+) seedlings at 5 and 8 wpi than Las (-) seedlings (Fig. 1a). In 2014, no significant effect of Las on *P.n.* infection incidence was detected at 11 wpi (Fig. 1b). *P.n.* infection incidence was not available at 8 wpi in the 2014 trial due to improperly prepared PARPH media.

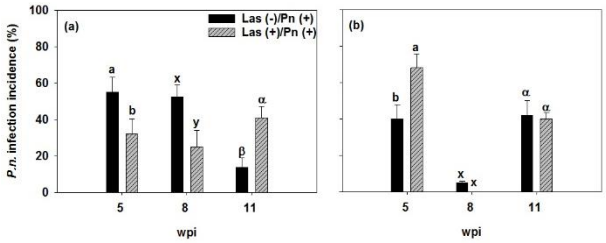

Fig 1. *Phytophthora nicotianae* (*P.n.*) infection incidence of Cleopatra mandarin seedlings at 5, 8 and 11 weeks post inoculation (wpi). (a) 2013 trial, (b) 2014 trial. Treatments: inoculation with *P.n.* (Las(-)/Pn(+)); co-inoculation of *Candidatus* Liberibacter asiaticus (Las) and *P.n.* (Las(+)/Pn(+)). Different letters denote significant difference at $P \leq 0.05$ within each harvest.

Fibrous root damage

Cleopatra mandarin root damage due to Las, *P.n.* and their interaction was assessed by measurement of fibrous root biomass at 5, 8 and 11 wpi. As expected, the non-inoculated seedlings continuously increased in root biomass in the 2013 and 2014 trials (Fig. 2a, b). No harvest time effect on root biomass of Las-infected, *P.n.*-infected, and co-inoculated seedlings was found, except that root biomass of Las-infected seedlings was greater at 5 wpi than at 8 and 11 wpi in the 2014 trial. At 5 wpi, Las significantly reduced fibrous root biomass in the 2013 trial, but not in the 2014 trial. In the 2013 trial, fibrous root biomass was 42%, 19% and 46% lower for the seedlings infected with Las alone, *P.n.* alone or both

together, respectively, compared to non-inoculated controls at 11 wpi (Fig. 2a). In the 2014 trial, root biomass was 51%, 29% and 39% lower for seedlings infected with Las, *P.n.* or both, respectively, compared to non-inoculated controls at 11 wpi (Fig. 2b). The combination of Las and *P.n.* caused similar root damage compared to Las or *P.n.* alone in either trial.

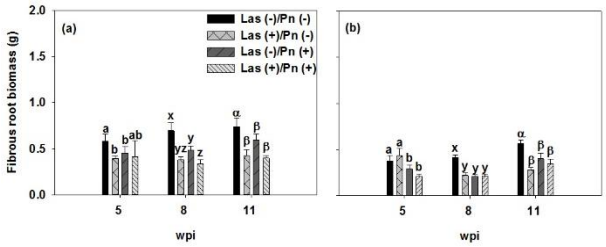

Fig 2. Fibrous root biomass of Cleopatra mandarin seedlings at 5, 8 and 11 weeks post inoculation (wpi). (a) 2013 trial, (b) 2014 trial. Treatments: Inoculation with *Candidatus* Liberibacter asiaticus (Las) (Las(+)Pn(-)), inoculation with *Phytophthora nicotianae* (*P.n.*) (Las(-)/Pn(+)); co-inoculation of Las and *P.n.* (Las(+)/Pn(+)); non-inoculated (Las(-)/Pn(-)). Different letters denote significant difference at $P \leq 0.05$ within each harvest.

Effect of Las on root starch concentration

Starch concentration in non-inoculated Cleopatra mandarin ranged from 34 to 97 mg per g root in the 2013 trial and 9 to 26 mg per g root in the 2014 trial. Starch concentration in non-inoculated and Las-infected roots fluctuated over time without following a clear progression. Control seedlings had a higher starch concentration in fibrous roots at 3 harvests than seedlings that were infected with Las (Fig. 3) in the 2013 trial, and at 5 and 11 wpi in the 2014 trial (Fig. 3).

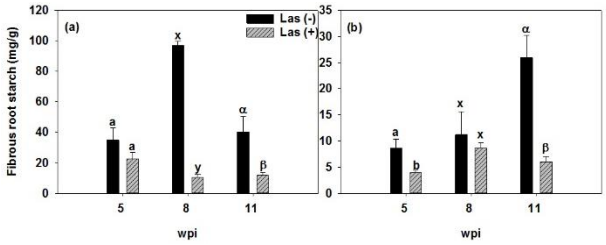

Fig 3. Fibrous root starch concentration of Cleopatra mandarin at 5, 8 and 11 weeks post root trimming (for *Phytophthora nicotianae* inoculation: wpi). (a) 2013 trial, (b) 2014 trial. Treatments: Inoculation with *Candidatus* Liberibacter asiaticus (Las) (Las (+)); non-inoculated (Las (-)). Different letters denote significant difference at $P \leq 0.05$ within each harvest.

Effect of Las on root sucrose concentration

Fibrous root sucrose of non-inoculated Cleopatra mandarin ranged from 8 to 30 and 12 to 20 mg per g root in the 2013 and 2014 trial, respectively (Fig. 4). Sucrose concentration in non-inoculated and Las-infected roots fluctuated over time without following a clear pattern. Las-infected roots had lower sucrose than non-inoculated roots at 5 and 11 wpi in the 2013 trial, and at 5 and 8 wpi in the 2014 trial.

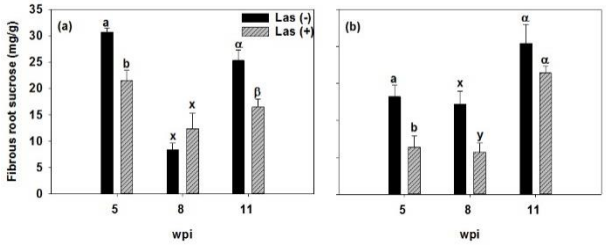

Fig 4. Fibrous root sucrose concentration of Cleopatra mandarin at 5, 8 and 11 weeks post root trimming (for *Phytophthora nicotianae* inoculation: wpi). (a) 2013 trial, (b) 2014 trial. Treatments: Inoculation with *Candidatus* Liberibacter asiaticus (Las) (Las (+)); non-inoculated (Las (-)). Different letters denote significant difference at $P \leq 0.05$ within each harvest.

Relationship between P.n. infection incidence and carbohydrate in fibrous roots

In both trials, the linear regression between *P.n.* infection incidence and carbohydrate concentration was positive for Las-infected roots and negative for seedlings not inoculated with Las (Fig. 5 and 6). The exception was at 8 wpi in the 2013 trial (Fig. 5) when seedlings infected with Las showed a negative linear relationship between *P.n.* infection incidence and carbohydrate concentration, whereas seedlings not inoculated with Las showed a positive relationship between *P.n.* infection incidence and carbohydrate concentration. The linear regression between *P.n.* infection incidence and carbohydrate concentration was not available at 8 wpi in the 2014 trial as *P.n.* was not recovered.

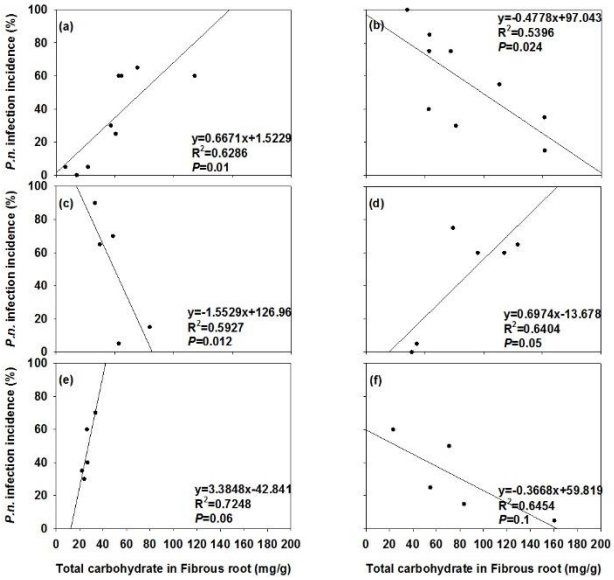

Fig 5. Cleopatra mandarin 2013. Linear regression of *Phytophthora nicotianae* (*P.n.*) infection incidence and Total available sugar at 5, 8 and 11 weeks post inoculation (wpi). Treatments: co-inoculation of *Candidatus* Liberibacter asiaticus (Las) and *P.n.* (Las(+)/Pn(+)); inoculation with *P.n.* (Las(-)/Pn(+)). 5 wpi: (a) Las(+)/Pn(+), (b) Las(-)/Pn(+); 8 wpi: (c) Las(+)/Pn(+), (d) Las(-)/Pn(+); 11 wpi: (e) Las(+)/Pn(+), (f) Las(-)/Pn(+). Points represent the value of 5-9 replicates.

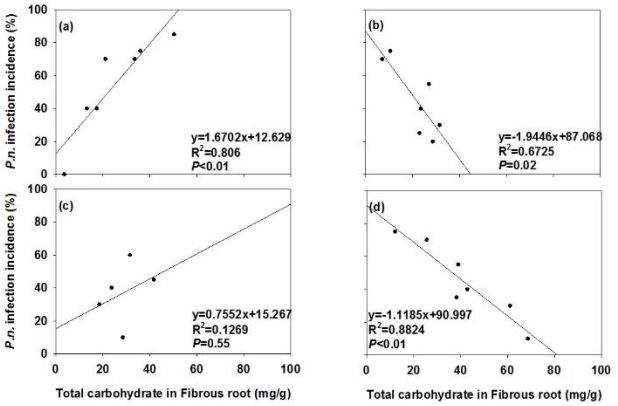

Fig 6. Cleopatra mandarin 2014. Linear regression of *Phytophthora nicotianae* (*P.n.*) infection incidence and Total available sugar at 5 and 11 weeks post inoculation (wpi). Treatments: co-inoculation of *Candidatus* Liberibacter asiaticus (Las) and *P.n.* (Las(+)/Pn(+)); inoculation with *P.n.* (Las(-)/Pn(+)). 5 wpi: (a) Las(+)/Pn(+), (b) Las(-)/Pn(+); 11 wpi: (c) Las(+)Pn(+), (d) Las(-)Pn(+). Points represent the value of 5-7 replicates.

Discussion

Ann et al. (2004) measured a reduction in plant height in asymptomatic Las-inoculated pummelo seedlings 6 months after Las inoculation compared to controls. However, fibrous root damage caused by Las was not evaluated. In this study, we confirmed that Cleopatra mandarin seedling fibrous roots can be damaged by Las infection before visible canopy symptoms develop (Johnson et al. 2014). Additionally, Ann et al. (2004) determined that Las and *P.n.* alone reduced height and biomass of sour orange 6 months after inoculation, and the combination of the 2 pathogens caused greater reduction than each one alone. In the present study, both pathogens reduced root biomass of Cleopatra mandarin seedlings. However, the combination of Las and *P.n.* did not significantly reduce the fibrous root biomass more than each root pathogen alone. This might be due to the small size of the seedlings. Compared to adult trees, seedling roots are more susceptible to *P.n.* infection and root loss (Graham 1995). Over 11 weeks, reduction of fibrous roots by either pathogen alone may have progressed to the point that viable roots were no longer available for further infection and damage by either pathogen. In the previous study (Ann et al. 2004), greater reduction in plant height and biomass caused by the combination of both pathogens than each alone 6 months after inoculation may have been an outcome of a longer-term interaction between Las and *P.n.* than we evaluated.

Las significantly increased or decreased *P.n.* infection incidence without a clear progression through time. Las lives in the phloem and *P.n.* infects the root cortex. The effect of Las on *P.n.* infection severity is probably mediated by citrus roots, the host for both pathogens. *P.n.* is a hemibiotroph, which requires living roots to infect and reproduce but can survive for some time in the absence of the host in dead roots or as resting spores (Panabières et al. 2016). Hence, a healthier root system provides more viable roots for *P.n.* to infect compared to a damaged HLB root system, whereas HLB roots are predisposed to *P.n.* infection (Ann et al. 2004). In the greenhouse evaluation process, we attempted to maintain the same environmental conditions during each trial and synchronize root developmental stage by root trimming before canopy symptoms developed. Nevertheless, it is difficult to sustain the same disease progress for the HLB root system because Las is distributed unevenly in the plant, and the infection time frame can vary from one inoculated seedling to another (Johnson et al. 2014). One explanation for the effect of Las on *P.n.* infection incidence at 5 wpi was reversed in 2013 and 2014 trials is that root loss in the 2014 trial was not as advanced as in the 2013 trial (Fig. 2). Ann et al. (2004) found that 14 to 35 days after Las inoculation, *P.n.* infection incidence was increased by Las indicating that Las promoted root susceptibility to *P.n.* infection. Johnson et al. (2014) detected Las in roots as early as 2 months after graft inoculation. Therefore, root loss probably had not occurred at 14 to 35 days after Las inoculation in the Ann et al. (2004) study. In our 2014 trial, root biomass loss was not detected at 5 weeks post root trimming. As Las infection progressed, root replacement and dieback occurred at the same time, and changing the proportion of viable to dead roots which complicated the progress of *P.n.* infection. Consistent with our greenhouse findings that Las interfered with *P.n.* infection, field surveys detected fluctuations of *P.n.* population in rhizosphere soil that follow seasonal and biennial changes as root damage of HLB trees progresses in Florida orchards (Graham and Feichtenberger 2015).

The interaction between Las and citrus roots revealed Las infection alters root quality and quantity. Lower starch and sucrose concentration in roots of Las-infected seedlings compared to non-infected control seedlings indicates a loss of root vitality which may account for the effect higher susceptibility of Las-infected fibrous roots to *P.n.* infection (Ann et al. 2004). Additionally, carbohydrate pools are an energy source for production of plant defense compounds. Jönsson (2006) proposed that elevation of carbohydrate concentration in *Quercus robur* increased tolerance to *P. quercina*. In this study, Cleopatra mandarin seedlings inoculated with *P.n.* with a higher carbohydrates (sucrose+starch) concentration had lower *P.n.* incidence (Fig. 5b, f; Fig. 6b, d), which is consistent with Jönsson's model that available carbohydrates in roots promotes tolerance to *Phytophthora* infection. In contrast, when Las was present, the tolerance of host associated with higher carbohydrates was not evidenced as higher carbohydrates were associated with greater *P.n.* infection incidence (Fig. 5a, e; Fig. 6a, c). However, at 8 wpi in the 2013 trial (Fig. 5d), higher carbohydrate did not reduce *P.n.* infection incidence as observed at other harvest times. This suggests that carbohydrate's role in plant defense to *P.n.* is more complex than it was proposed in the model of *Quercus robur* and *P. quercina*. Furthermore, Las disrupted host carbohydrate status which might mediate the interaction between host and *P.n.*

This study was conducted to gain further insight into how HLB affects the fibrous root system of citrus trees before canopy symptoms develop and how Las interacts with root pathogens. As HLB progresses, phloem blockage associated with foliar symptoms disrupts carbohydrate allocation to the roots, which could alter the interaction between Las, *P.n.* and the host as root physiology is changed. More in-depth investigation of root physiological responses is needed to fully understand the interaction between Las and *P.n.* as foliar symptoms progress.

These findings confirm that to maintain function of Las-infected roots for nutrient and water uptake it is important to minimize stress from biotic and abiotic interactions in the rhizosphere. If damaging populations of *Phytophthora* are detected, fungicide application can be considered to reduce damage to roots by *P.n.* However, since Las causes comparable damage to roots, controlling *P.n.* infection may provide only marginal benefit for sustaining HLB fibrous root health (Graham and Feichtenberger 2015).

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References

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.

Ann PJ, Ko WH, Su HJ. 2004. Interaction between Likubin bacterium and *Phytophthora parasitica* in citrus hosts. Eur J Plant Pathol. 110:1-6.

Bassanezi RB, Montesino LH, Gasparoto MCG, Filho AB, Amorim L. 2011. Yield loss caused by huanglongbing in different sweet orange cultivars in São Paulo, Brazil. Eur J Plant Pathol. 130: 577-586.

Bassanezi RB, Montesino LH, Stuchi ES. 2009. Effects of huanglongbing on fruit quality of sweet orange cultivars in Brazil. Eur J Plant Pathol. 125:565-572.

Bové JM. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J Plant Pathol. 88:7-37.

da Graca JV. 1991. Citrus greening disease. Annu Rev Phytopathol. 29:109-136.

Davis RM, Menge JA. 1981. *Phytophthora parasitica* inoculation and intensity of vesicular-arbuscular mycorrhizae in citrus. New Phytol. 87:705-715.

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 P. 74:76-83.

Folimonova SY, Robertson CJ, Garnsey SM, Gowda S, Dawson WO. 2009. Examination of the responses of different genotypes of citrus to Huanglongbing (citrus greening) under different conditions. Phytopathology. 99:1346-1354.

Garnier M, Bové JM. 1983. Transmission of the organism associated with citrus greening disease from sweet orange to periwinkle by dodder. Phytopathology. 73:1358-1363.

Gottwald TR. 2010. Current epidemiological understanding of citrus huanglongbing. Annu Rev Phytopathol. 48:119-139.

Graham JH. 1995. Root regeneration and tolerance of citrus rootstocks to root rot caused by *Phytophthora nicotianae*. Phytopathology. 85:111-117.

Graham JH, Feichtenberger E. 2015. Citrus *Phytophthora* diseases: Management challenges and successes. J Cit Pathol. iocv_journalcitruspathology_27203.

Graham JH, Irey M, Taylor J. 2011. *Phytophthora* damage to roots: A potential contributor to decline of HLB-affected trees. Citrus Ind. 92:20-23.

Graham JH, Johnson E, Gottwald TR, Irey MS. 2013. Presymptomatic fibrous root decline in citrus trees caused by Huanglongbing and potential interaction with *Phytophthora* spp. Plant Dis. 97:1195-1199.

Halbert SE, Manjunath KL. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Fla Entomol. 87:330–353.

Jagoueix S, Bové JM, Garnier M. 1994. The phloem-limited bacterium of greening disease of citrus is a member of the alpha subdivision of the Proteobacteria. Int J Syst Evol Micr. 44:379-386.

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. 63:290-298.

Jönsson U. 2006. A conceptual model for the development of *Phytophthora* disease in *Quercus robur*. New Phytol. 171:55-67.

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. 99:50-57.

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. 249:687-697.

Panabières F, Ali GS, Allagui MB, Dalio RJ, Gudmestad NC, Kuhn ML, Roy SG, Schena L, Zampounis A. 2016. *Phytophthora nicotianae* diseases worldwide: new knowledge of a long-recognised pathogen. Phytopathol Mediterr. 55:20-40.

Rosales R, Burns JK. 2011. Phytohormone changes and carbohydrate status in sweet orange fruit from Huanglongbing-infected trees. J Plant Growth Regul. 30: 312-321.

Schneider H. 1968. Anatomy of greening-diseased sweet orange shoots. Phytopathology. 58:1155-1160.

Trivedi P, Sagaram US, Kim JS, Brlansky RH, Rogers ME, Stelinski LL, Oswalt C, Wang N. 2009. Quantification of viable *Candidatus* Liberibacter asiaticus in hosts using quantitative PCR with the aid of ethidium monoazide (EMA). Eur J Plant Pathol. 124:553-563.

Tsao PH. 1971. Chlamydospore formation in sporangium-free liquid cultures of *Phytophthora parasitica*. Phytopathology. 6:1412-1413.

Wang Z, Yin Y, Hu H, Yuan Q, Peng G, Xia Y. 2006. Development and application of molecular based diagnosis for '*Candidatus* Liberibacter asiaticus', the causal pathogen of citrus huanglongbing. Plant Pathol. 55:630-638.