



# Influence of rootstock variety on Huanglongbing disease development in field-grown sweet orange (*Citrus sinensis* [L.] Osbeck) trees

Ute Albrecht\*, Greg McCollum, Kim D. Bowman

U.S. Horticultural Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, 2001 South Rock Road, Fort Pierce, FL 34945, USA

## ARTICLE INFO

### Article history:

Received 2 December 2011

Received in revised form 16 February 2012

Accepted 21 February 2012

### Keywords:

Citrus greening  
Disease resistance  
Rootstock

## ABSTRACT

Huanglongbing (HLB), a bacterial disease of citrus, is causing substantial economic losses to the citrus industry worldwide. Sweet oranges are highly susceptible to the disease, and account for nearly 90% of all varieties grown in Florida. Rootstock is an important component of commercial citrus production, and tolerance to HLB has been reported for some rootstock varieties. Our objective was to investigate if rootstock selection has an effect on HLB disease development under natural conditions in the field. Four field trials with sweet orange scion on 15 different rootstocks were evaluated for incidence of *Candidatus Liberibacter asiaticus* (Las), the suspected causal agent of HLB, as well as incidence of foliar disease symptoms, canopy damage, and stem growth during the first years after Las began to spread into the trials. Trials ranged from two to nine years in age and included hybrids of trifoliolate orange along with other rootstocks standard for citrus production in Florida. Fruit yield and soluble solids content were analyzed for the oldest trial. Our study showed that rootstock did not affect disease incidence and that trees on all rootstocks were considerably damaged by HLB. However, tolerance to HLB was higher in trees grafted on some rootstock selections. In the youngest trial, stem diameters on Volkamer lemon increased 53% while trees on US-852, Benton citrange and Swingle citrumelo grew the least at 21–26% from 2008 to 2010. Depending on trial and time of observation, foliar HLB symptoms on US-897 were less than on many other rootstocks, with ranks of 3.3–4.0 compared with 4.5–4.9 observed for US-812, US-852, Carrizo citrange and Kinkoji. Canopy damage ranks were 1.4–2.6 for trees on US-802 compared with 3.9–4.1 for trees on US-812, U-852, Sour orange, and Swingle in the oldest trial. In the youngest trials, trees on Volkamer showed least canopy damage while trees on US-852 and Benton were more affected. Highest fruit yields of 30–64 kg/tree were obtained from trees on US-802 and Carrizo. In 2010, highest juice soluble solids content of 2.8–2.9 kg was observed for trees on US-897 and US-812.

Published by Elsevier B.V.

## 1. Introduction

Citrus is the most important tree fruit crop in the world with production in over 100 countries on all six continents (Saunt, 2000). Among the many diseases of citrus, Huanglongbing (HLB) is possibly the most destructive one, generating substantial economic losses to the industry in affected areas. HLB probably originated in India in the 1700s, before spreading to China where a first published account did not emerge until 1956 (Lin, 1956; Gottwald, 2010). The disease has now spread to most citrus-growing areas worldwide, including Asia, Saudi Arabia, Africa, and the Americas (Bové, 2006). HLB was found in Florida in 2005 (Halbert, 2005), just one year after its first discovery in the Western Hemisphere in Sao Paula, Brazil (Teixeira et al., 2005).

The Chinese name Huanglongbing refers to the appearance of yellow shoots in the canopy of diseased trees, which are the result of an asymmetric blotchy mottling of older leaves or severe chlorosis, often resembling zinc- or other nutritional deficiencies (McClean and Schwarz, 1970). Fruit from HLB-affected trees are often small or misshapen and do not color properly, for which reason the disease is also often referred to as citrus greening. HLB symptoms appear to be associated with plugging of sieve pores, leading to phloem necrosis and blockage of the translocation stream (Schneider, 1968; Achor et al., 2010). Transcriptional analyses of HLB-affected citrus discovered major changes in expression patterns of genes associated with carbohydrate metabolism and other metabolic pathways (Albrecht and Bowman, 2008; Kim et al., 2009; Fan et al., 2010). Progression of the disease eventually leads to fruit drop and twig dieback, resulting in tree decline and often rendering a tree non-productive within few years. No known cure for HLB exists at present and chemical insect control, nutritional applications, removal of infected trees, and the establishment of pathogen-free nursery systems are the most common management strategies.

\* Corresponding author. Tel.: +1 772 462 5941; fax: +1 772 462 5986.  
E-mail address: [ute.albrecht@ars.usda.gov](mailto:ute.albrecht@ars.usda.gov) (U. Albrecht).

The suspected causal agents of HLB are phloem-inhabiting gram-negative bacteria, which are difficult to maintain in pure culture (Davis et al., 2008; Sechler et al., 2009). Three different bacterial species are associated with HLB. *Candidatus Liberibacter asiaticus* is the geographically most widespread species and is found in all HLB-affected countries except Africa (Bové, 2006). *Ca. L. africanus* is so far restricted to Africa (Garnier et al., 2000), whereas *Ca. L. americanus*, which was restricted to Brazil until recently (Teixeira et al., 2005) has now been reported in China (Lou et al., 2010).

Transmission of liberibacters occurs through insect vectors, the Asian citrus psyllid *Diaphorina citri* Kuwayama or the African citrus psyllid *Trioza erytrea* Del Guercio. A phytoplasma associated with disease symptoms indistinguishable from HLB was identified in citrus growing areas in São Paulo State, Brazil (Teixeira et al., 2008). The vector involved in the transmission of this phytoplasma has not been identified thus far. In addition to the psyllid vectors, other modes of transmission for liberibacters to citrus include dodder (*Cuscuta* sp.) and grafting with diseased budwood (Halbert and Manjunath, 2004). Recent studies indicate no evidence of a vertical transmission of the bacteria from citrus seed to seedling (Albrecht and Bowman, 2009; Hartung et al., 2010).

HLB affects all known citrus species and citrus relatives with little known resistance (McClellan and Schwarz, 1970; Miyakawa and Zhao, 1990; Folimonova et al., 2009; Shokrollah et al., 2009). Among commercial citrus varieties, sweet oranges (*Citrus sinensis* L.), mandarins (*C. reticulata* Blanco) and tangelos (hybrids of *C. reticulata*) are most susceptible to HLB. An important component of commercially grown citrus trees is the rootstock, which may determine success or failure of a citrus operation. Rootstock selection is based on tolerance to pests, diseases, soil conditions, and cold, as well as on the desired effect on scion vigor, fruit size, fruit quality, and yield (Castle, 2010). Hybrids of trifoliolate orange (*Poncirus trifoliata* L. Raf.), such as Swingle citrumelo (*C. paradisi* Macf. x *P. trifoliata*) and Carrizo citrange (*C. sinensis* x *P. trifoliata*), are among the most commonly used rootstocks for production of sweet oranges in Florida (2010 Annual Report, Bureau of Citrus Budwood Registration). Conflicting results have been reported regarding HLB disease development in trifoliolate-type genotypes. Nariani (1981) observed moderate to severe HLB symptoms in citranges and *P. trifoliata*, while McClellan and Schwarz (1970) did not observe any well-defined leaf symptoms. No distinct HLB symptoms were observed on *P. trifoliata* in a study by Miyakawa (1980), and Folimonova et al. (2009) reported inconsistent symptoms for *P. trifoliata*. A recent report describes tolerance of the trifoliolate hybrid US-897 (*C. reticulata* x *P. trifoliata*) to HLB (Albrecht and Bowman, 2011), although the effect of US-897 on HLB disease development in the scion was not investigated.

A small number of studies examined the effect of rootstock on the performance of citrus trees in the presence of HLB. These studies were mainly restricted to India (Nariani, 1981; Cheema et al., 1982) and South Africa (Van Vuuren and Moll, 1985). Little is known regarding the effects of rootstocks on HLB disease development in scions typically used for commercial citrus production on the American continent. The USDA citrus breeding program is one of the oldest of its kind, dating back to 1893, and several field trials have been established at the USDA Picos Farm in Ft. Pierce, Florida, evaluating new rootstock selections along with standard rootstock varieties. HLB was detected at the Picos Farm soon after the discovery of HLB in Florida in 2006. The disease is now endemic at the Picos Farm and other nearby locations. The purpose of this study was to investigate whether rootstock type influences HLB disease development in sweet orange scions under natural conditions in the field. Based on published reports of tolerance observed in the genus *Poncirus*, we hypothesized to find a better performance of trees on some of the trifoliolate hybrid genotypes. Four trials consisting of sweet orange scion on 15 different rootstocks were evaluated for

Las incidence, HLB foliar disease symptoms, canopy damage, and stem growth. Data on fruit yield and soluble solids content were studied in one trial.

## 2. Materials and methods

### 2.1. Field trials

Rootstock liners were budded with certified disease-free 'Valencia'- or 'Early Gold' sweet orange (*C. sinensis*) budwood from the Bureau of Citrus Budwood Registration (Florida Department of Agriculture & Consumer Services, Division of Plant Industry) at six to eight months of age by the inverted T budding method. Rootstock varieties included hybrids of mandarin and 'Pummelo' with trifoliolate orange as well as citranges, lemons, mandarins, and other standard rootstocks (Table 1). Grafted plants were maintained in the USHRL greenhouses for 8–12 months until suitable for field planting.

Trial 1 ('Valencia') was established in 1999 in six rows of trees with 10 trees per rootstock variety. Trial 2 ('Early Gold') was established in 2002 in six rows of trees with 18 trees per rootstock variety. Trial 3 ('Valencia') was established in 2004 in ten rows of trees with 20 trees per rootstock variety. Trial 4 ('Valencia') was established in 2006 in six rows of trees with 12 trees per rootstock variety. All trees were planted at a spacing of either 3 m x 8 m or 5 m x 8 m, with one or more border trees at the end of each row. Trials were arranged as randomized complete block designs. Some trees were lost during the establishment phases or as a result of hurricanes in 2004, reducing the number of trees on some rootstocks (Table 1). Trials 1, 2 and 4 were conducted at the USHRL-USDA (Picos) Farm in St. Lucie County, Florida. Trial 3 was located 1.5 km west of the Picos Farm and immediately South of a citrus grove which had been abandoned prior to 2004. Trees were irrigated by micro sprinklers three times a week in the absence of adequate rain fall. Fertilizer and pesticides were applied seasonally or as needed, but did not include increased foliar nutritional applications (<http://edis.ifas.ufl.edu/hs1165>) or aggressive psyllid control as currently practiced in many HLB-affected commercial citrus groves in Florida.

### 2.2. Detection of *Ca. L. asiaticus* (*Las*)

For each trial six to eight leaves per tree were collected from different locations in the tree canopy in spring 2008, fall 2008 and spring 2009. Petioles and midribs were ground in liquid nitrogen with a mortar and pestle and 100 mg of ground tissue was used for DNA extraction. DNA was extracted using the Plant DNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. For the detection of *Las*, polymerase chain reactions (PCR) were performed with the isolated DNA as described in Albrecht and Bowman (2009).

### 2.3. Foliar disease symptoms and canopy damage

Trees in each trial were evaluated for HLB symptoms in spring 2008, fall 2008, spring 2009, and fall 2009. HLB symptoms were ranked on a scale in which 1 = no apparent symptoms, 2 = symptoms restricted to less than five leaves (typically on one terminal shoot), 3 = symptoms visible on five or more leaves on one or more branches, but on less than 10% of branches, 4 = symptoms visible on 10–30% of branches, 5 = symptoms visible on 30–60% of branches, and 6 = symptoms visible on more than 60% of branches. Symptoms included chlorosis, often in combination with reduced leaf size and typically restricted to terminal shoots, and blotchy mottle, which was visible on terminal shoots as well as on more proximal parts of branches. Canopy damage was ranked on a scale in which 1 = full

**Table 1**  
Rootstocks and number of trees evaluated in four sweet orange field trials conducted in St. Lucie County, FL.

Rootstock	Scientific name	Number of trees			
		Trial 1 (1999) <sup>a</sup>	Trial 2 (2002)	Trial 3 (2004)	Trial 4 (2006)
US-801	<i>C. reticulata</i> Blanco 'Changsha' x <i>P. trifoliata</i> (L.) Raf. 'English Small trifoliolate orange'	10	18	–	–
US-802	<i>C. grandis</i> (L.) Osb. 'Siamese pummelo' x <i>P. trifoliata</i> 'Gotha Road' trifoliolate orange	10	–	20	12
US-812	<i>C. reticulata</i> 'Sunki' x <i>P. trifoliata</i> 'Benecke'	10	18	19	12
US-852	<i>C. reticulata</i> 'Changsha' x <i>P. trifoliata</i> 'English Large trifoliolate orange'	10	–	17	12
US-897	<i>C. reticulata</i> 'Cleopatra' x <i>P. trifoliata</i> 'Flying Dragon'	9	18	19	12
US-942	<i>C. reticulata</i> 'Sunki' x <i>P. trifoliata</i> 'Flying Dragon'	10	18	20	12
Benton citrange	<i>C. sinensis</i> (L.) Osb. x <i>P. trifoliata</i>	–	–	20	12
Carrizo citrange	<i>C. sinensis</i> x <i>P. trifoliata</i>	10	18	–	–
Cleopatra mandarin	<i>C. reticulata</i> Blanco	–	–	19	12
Kinkoji	<i>C. obovoidea</i> Takahashi	–	18	20	12
Rough lemon	<i>C. jambhiri</i> Lushington	9	–	–	–
Sour orange	<i>C. aurantium</i> L.	10	–	20	12
Sun Chu Sha	<i>C. reticulata</i>	9	18	–	12
Swingle citrumelo	<i>C. paradisi</i> Macf. x <i>P. trifoliata</i>	10	–	19	12
Volkamer lemon	<i>C. volkameriana</i> Ten. & Pasq.	–	18	20	12
Total number of trees		107	144	213	144

<sup>a</sup> Dates of trial establishment are in parentheses.

tree canopy and little die-back, 2 = dieback/defoliation in less than 10% of the canopy, 3 = dieback/defoliation in 10–30% of the canopy, 4 = dieback/defoliation in 30–60% of the canopy and/or stunting of the tree, and 5 = dieback/defoliation in more than 60% of the canopy and/or severe stunting of the tree.

#### 2.4. Scion- and rootstock growth

Scion- and rootstock diameters of trees in all four trials were measured 5 cm above and 5 cm below the graft union, respectively, using a digital caliper. Measurements were conducted in spring/summer of 2006, 2008 and 2010 and growth was expressed as percent increase of mean stem diameters from 2006 to 2008 and from 2008 to 2010.

#### 2.5. Fruit yield and juice soluble solids

Yields for trial 1 were measured in standard 40.8 kg boxes. Harvest dates were June 2008 and 2009. Soluble solids for trial 1 were measured in April 2008, April 2009, and May 2010 on fruit samples consisting of 24 randomly collected fruit per tree. Soluble solids were determined as the product of percentage juice and percentage sugar (°Brix) per box of fruit.

#### 2.6. Statistical analyses

Based on PCR results, a putative time of infection with *Las* – by spring 2008 (early) or after spring 2008 (late) – was assigned to each tree. This differentiation into early and late infected trees allowed us to gain information on the short-term effects of infection on citrus trees in a natural setting, despite the non-controllable and rapid spread of *Las* in infected citrus groves. Analysis of variance (ANOVA) was used to determine the effects of rootstock and time of infection on scion- and rootstock growth and on fruit quality and -yield. Significant ANOVA tests were followed by multiple comparisons of means using Tukey's HSD (Honestly Significant Difference) procedure. Kruskal–Wallis ANOVA of ranks was performed to determine the effects of rootstock and time of infection on leaf symptom expression and canopy damage, followed by Dunn's post test. Field trials were analyzed as completely randomized designs. Analyses were performed using STATISTICA version 6.0 (StatSoft,

Tulsa, OK). Dunn's post test was performed using GraphPad Prism version 4.03 (GraphPad Software, Inc., San Diego, CA).

### 3. Results

#### 3.1. Percentage of trees PCR-positive for *Las*

PCR analysis identified 39% of trees in trial 1 as positive for *Las* in spring 2008 (Table 2). Incidence of infection was lowest (20–22%) in trees on Carrizo citrange, Sour orange and Rough lemon and highest (56–60%) in trees on US-801, US-897 and Sun Chu Sha. The percentage of infected trees for trial 2 was 49% on average and ranged from 39% in trees on Kinkoji to 67% in trees on Volkamer lemon. Percentage of infection for trial 3 ranged from 42% on US-812 to 79% on Swingle citrumelo with an average of 62%. An average of 42% of trees was identified as PCR-positive in trial 4. Incidence of infection was lowest on US-812, Sun Chu Sha and Sour orange (15–25%) and highest on Benton citrange (75%). In fall of the same year, nearly all plants were infected with *Las*. By spring 2009 the percentage of infection was 100% in all trials.

#### 3.2. Foliar HLB symptom expression

Average HLB symptom ranks in spring 2008 ranged from 1.8 in trial 1, to 3.6 in trial 4 (Table 3). Symptom ranks were significantly ( $P < 0.05$ ) higher in trees PCR-positive for *Las* compared with PCR-negative trees, with the highest rank observed in the most recently established trial 4 (5.4). In fall 2008, average symptom ranks varied from 3.5 in trial 1 to 5.8 in trial 4. Except for trial 4, where symptoms were very abundant independent of the time of infection, ranks were significantly higher in trees infected earlier than in trees infected later. Average symptom ranks in spring 2009 ranged from 3.0 in trial 1 to 5.2 in trial 4 and time of infection was significant for trials 1 and 4. In fall 2009, average ranks were 4.6–5.9 and time of infection was significant for trials 2 and 3. HLB symptom expression was not significantly ( $P > 0.05$ ) affected by rootstock in 2008. In spring 2009, trees on US-897 had significantly lower symptom ranks in trial 2 (4.0) compared with Carrizo and US-812 (4.9) and in trial 3 (3.3) compared with Kinkoji and US-852 (4.5–4.6). In fall 2009, rootstock did not significantly affect leaf symptom

**Table 2**Percentage of sweet orange trees on different rootstocks PCR-positive for *Ca. Liberibacter asiaticus* in spring and fall of 2008.

Rootstock	Trial 1 (1999) <sup>a</sup>		Trial 2 (2002)		Trial 3 (2004)		Trial 4 (2006)	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
US-801	60	100	50	100	–	–	–	–
US-802	40	100	–	–	55	100	58	100
US-812	30	100	50	100	42	95	15	100
US-852	50	100	–	–	65	100	33	100
US-897	56	100	44	100	58	89	58	100
US-942	40	100	50	100	70	100	42	100
Benton citrange	–	–	–	–	55	95	75	100
Carrizo citrange	20	100	44	100	–	–	–	–
Cleopatra mandarin	–	–	–	–	68	89	50	100
Kinkoji	–	–	39	100	60	90	42	100
Rough lemon	22	100	–	–	–	–	–	–
Sour orange	20	90	–	–	65	80	25	100
Sun Chu Sha	56	100	50	100	–	–	25	100
Swingle citrumelo	40	100	–	–	79	100	50	100
Volkamer lemon	–	–	67	100	65	95	33	100
Average	39	99	49	100	62	94	42	100

<sup>a</sup> Dates of trial establishment are in parentheses.

expression except in trial 2 where lowest ratings (4.3) were observed for trees on US-897.

### 3.3. Canopy damage

Average canopy damage ranks in 2008 ranged from 2.2 in trial 1 to 3.9 in trial 2 (Table 4). Canopy damage was significantly ( $P < 0.05$ ) higher in earlier infected trees than in later infected trees. In 2009, average ranks ranged from 3.4 in trial 1 to 4.7 in trial 4. In spring 2009, with the exception of trial 3, canopies were significantly more damaged in trees infected earlier compared with trees infected later. In fall 2009, time of infection was significant only for trial 2. The effect of rootstock on canopy damage was significant depending on the time of observation. In trial 1, canopies of trees on US-802 displayed least damage throughout the study, and significantly lower ranks (2.6) were observed for US-802 compared with US-852 and Swingle (4.0) in spring 2009 and compared with US-812, US-852, Sour orange and Swingle (3.9–4.1) in fall 2009. In trial 2, lowest ranks were observed for trees on US-801 and Sun Chu Sha in fall 2008; however, post hoc tests were not significant ( $P > 0.05$ ). In trial 3, canopy damage for trees on Volkamer lemon was lowest throughout the study with average ranks not exceeding 3.1. Highest ranks (4.0–4.2), and thus most damage, were observed for trees on US-852, Swingle citrumelo and Benton citrange in 2009. Ranks for trees on Volkamer lemon were also lowest (2.3–4.2) in trial 4 throughout the study, although differences between rootstocks were significant only for spring 2009. Trees on US-852 performed consistently poorest in this trial.

### 3.4. Scion- and rootstock growth

Scion stem diameters of trees in trial 1 increased on average 9.6% from 2006 to 2008 and 5.9% from 2008 to 2010 (Table 5). Growth was not significantly ( $P > 0.05$ ) affected by rootstock or time of infection with *Las*. Rootstock stem diameters increased 9.2% and 10.2% on average from 2006 to 2008 and from 2008 to 2010, respectively. Rootstock growth did not vary significantly between rootstock varieties, but from 2008 to 2010 was significantly less in trees infected earlier (8.9%) compared with trees infected later (11.1%).

Scion stem diameters increased 38% on average from 2006 to 2008 in trial 2 and growth varied significantly depending on rootstock and time of infection. Scions on Kinkoji increased significantly more (49%) in diameter compared with scions on US-897, US-942, US-801, US-812, and Carrizo, which grew 29–36% on

average. Trees infected earlier grew significantly less (34%) than trees infected later (41%). From 2008 to 2010 the average percentage of scion growth was 14% and most growth was observed for trees on US-897 (17.9%). Time of infection did not significantly affect scion growth. Rootstock diameters increased 37% and 17% on average from 2006 to 2008 and from 2008 to 2010, respectively, and growth differed significantly among rootstocks and time of infection. Most growth was observed for Kinkoji and Sun Chu Sha from 2006 to 2008 (44–46%) and for Sun Chu Sha and US-897 from 2008 to 2010 (20–21%). Rootstock growth of earlier infected trees was 33% from 2006 to 2008 and 14.6% from 2008 to 2010, compared with later infected trees which grew 41% and 19% on average.

In trial 3, scion diameters increased 77% on average from 2006 to 2008 and growth was significantly affected by rootstocks and time of infection. Trees on US-812 grew significantly more (92%) compared with Benton citrange (63%). Trees infected earlier with *Las* grew 71% while trees infected later grew 86%. Scion diameters increased by 32% on average from 2008 to 2010 and growth was greatest in trees on Cleopatra mandarin (38%). Time of infection significantly affected scion growth, which was 30% in trees infected earlier compared with 35% in trees infected later. From 2006 to 2008 rootstock diameters increased 73% on average, with most growth observed for US-812, Cleopatra mandarin and Volkamer lemon (82–84%). Increase in rootstock diameter was significantly lower in earlier infected trees (69%) compared with later infected trees (80%). The average percentage of rootstock growth from 2008 to 2010 was 32% and did not vary significantly between rootstocks and time of infection.

Scion stem diameters increased 116% on average from 2006 to 2008 in trial 4, and growth was significantly affected by rootstock and time of infection. Scions on Sour orange grew the most (147%), followed by Volkamer lemon (135%). The smallest increase in stem diameter was on Kinkoji (91%). From 2008 to 2010 scion diameter increased 33% on average and trees on Volkamer lemon grew significantly more (53%) than trees on US-852, Benton citrange and Swingle citrumelo (21–26%). Time of infection did not significantly affect scion growth. Rootstock diameters increased 112% on average from 2006 to 2008 and Sour orange grew significantly more (148%) than US-802, Kinkoji, Swingle, US-942, Sun Chu Sha, and Cleopatra mandarin (96–107%). Rootstocks from trees infected earlier grew significantly less (99%) than trees infected later (121%). From 2008 to 2010 rootstock diameters increased 29% on average and Volkamer lemon grew significantly more (45%) than US-852 (19%). Although a significant interaction was observed for

**Table 3**  
Foliar HLB symptom expression in sweet orange trees on different rootstocks from spring 2008 to fall 2009.

Rootstock	HLB symptom rank <sup>a</sup>							
	Spring 2008		Fall 2008		Spring 2009		Fall 2009	
<i>Trial 1 ('Valencia', 1999)</i>								
US-801	2.2	a	3.8	a	3.1	a	4.8	a
US-802	1.7	a	3.3	a	3.0	a	5.0	a
US-812	1.5	a	3.5	a	3.3	a	5.0	a
US-852	2.0	a	4.0	a	3.4	a	5.0	a
US-897	2.6	a	3.6	a	3.0	a	4.8	a
US-942	1.9	a	3.3	a	3.0	a	4.8	a
Carrizo citrange	1.6	a	2.9	a	2.5	a	4.7	a
Rough lemon	1.6	a	3.7	a	3.2	a	5.2	a
Sour orange	1.7	a	2.6	a	3.2	a	5.0	a
Sun Chu Sha	1.7	a	3.9	a	2.8	a	5.1	a
Swingle citrumelo	1.9	a	4.2	a	3.1	a	5.1	a
Average	1.8		3.5		3.0		4.9	
Early infected <sup>b</sup>	3.0		4.5		3.7		5.0	
Late infected <sup>c</sup>	1.1		2.9		2.6		4.9	
Time of infection	$P < 0.0001$		$P < 0.0001$		$P < 0.0001$		ns	
Rootstock	ns		ns		ns		ns	
<i>Trial 2 ('Early Gold', 2002)</i>								
US-801	3.0	a	5.0	a	4.6	ab	4.8	a
US-812	3.5	a	5.2	a	4.9	a	4.6	a
US-897	2.7	a	4.8	a	4.0	b	4.3	a
US-942	3.7	a	5.1	a	4.4	ab	4.4	a
Carrizo citrange	3.8	a	5.7	a	4.9	a	4.7	a
Kinkoji	3.4	a	5.4	a	4.6	ab	4.7	a
Sun Chu Sha	3.4	a	4.9	a	4.7	ab	4.9	a
Volkamer lemon	3.8	a	5.5	a	4.7	ab	4.6	a
Average	3.4		5.2		4.6		4.6	
Early infected	4.4		5.6		4.5		4.4	
Late infected	2.5		4.8		4.6		4.9	
Time of infection	$P < 0.0001$		$P < 0.0001$		ns		$P < 0.0001$	
Rootstock	ns		ns		$P < 0.0172$		$P < 0.0342$	
<i>Trial 3 ('Valencia', 2004)</i>								
US-802	2.4	a	4.1	a	4.4	ab	5.1	a
US-812	2.6	a	4.1	a	4.1	ab	4.8	a
US-852	3.8	a	4.4	a	4.6	a	5.2	a
US-897	3.4	a	3.9	a	3.3	b	4.9	a
US-942	3.5	a	4.9	a	4.3	ab	5.1	a
Benton citrange	3.2	a	4.1	a	4.1	ab	5.0	a
Cleopatra mandarin	3.3	a	4.1	a	4.1	ab	5.4	a
Kinkoji	2.9	a	4.6	a	4.5	a	5.2	a
Sour orange	3.2	a	3.6	a	3.5	ab	5.2	a
Swingle citrumelo	3.8	a	4.9	a	3.9	ab	5.1	a
Volkamer lemon	3.1	a	4.3	a	4.2	ab	5.1	a
Average	3.2		4.3		4.1		5.1	
Early infected	4.4		5.2		4.1		5.0	
Late infected	1.3		2.7		4.0		5.3	
Time of infection	$P < 0.0001$		$P < 0.0001$		ns		$P < 0.0001$	
Rootstock	ns		ns		$P < 0.0020$		ns	
<i>Trial 4 ('Valencia', 2006)</i>								
US-802	4.0	a	6.0	a	4.8	a	6.0	a
US-812	2.5	a	5.9	a	4.8	a	5.8	a
US-852	3.9	a	6.0	a	5.5	a	6.0	a
US-897	4.3	a	5.8	a	5.0	a	5.8	a
US-942	4.0	a	5.4	a	4.6	a	6.0	a
Benton citrange	4.2	a	6.0	a	5.5	a	5.9	a
Cleopatra mandarin	4.1	a	5.4	a	5.3	a	5.9	a
Kinkoji	3.5	a	6.0	a	5.7	a	5.9	a
Sour orange	2.7	a	6.0	a	5.3	a	5.8	a
Sun Chu Sha	3.5	a	5.9	a	5.5	a	6.0	a
Swingle citrumelo	3.3	a	5.9	a	5.3	a	5.9	a
Volkamer lemon	2.8	a	5.8	a	4.8	a	5.8	a
Average	3.6		5.8		5.2		5.9	
Early infected	5.4		5.9		4.8		5.8	
Late infected	2.2		5.8		5.4		6.0	

Table 3 (Continued)

Rootstock	HLB symptom rank <sup>a</sup>			
	Spring 2008	Fall 2008	Spring 2009	Fall 2009
Time of infection	$P < 0.0001$	ns	$P < 0.0050$	ns
Rootstock	ns	ns	ns	ns

<sup>a</sup> Foliar HLB symptoms were ranked from 1 (no symptoms) to 6 (symptoms visible on more than 60% of branches). Symptoms included chlorosis, often in combination with reduced leaf size, and blotchy mottle of leaves.

<sup>b</sup> PCR-positive for *Ca. L. asiaticus* by spring 2008.

<sup>c</sup> PCR-positive for *Ca. L. asiaticus* after spring 2008. Significant differences were determined using Kruskal-Wallis ANOVA of ranks with  $P < 0.05$  considered as not significant (ns). Numbers in each column followed by different letters are significantly different according to Dunn's post test ( $P < 0.05$ ).

rootstock and time of infection, rootstock growth was not significantly reduced in trees which became later infected with *Las*.

### 3.5. Fruit yield and juice soluble solids

In 2008, the average yield from trees in trial 1 was 40 kg and was significantly affected by rootstock (Table 6). Trees on US-802 and on Carrizo citrange produced highest yields of 63–64 kg. Lowest yields ranged from 27 kg for trees on US-897 to 35 kg for trees on US-801 and US-852. Yield was not affected by the time of infection with *Las*. In 2009, the average yield was 22 kg and, despite being highest in US-802 and Carrizo (30–31 kg), did not vary significantly between rootstocks or time of infection. Juice soluble solids content was 3.0 kg per box on average in 2008 and varied significantly between rootstocks. Soluble solids were highest for fruit on US-812 (3.3 kg) followed by Swingle citrumelo (3.2 kg), while the lowest solids were obtained from fruit on Rough lemon (2.7 kg). Trees infected earlier with *Las* had significantly less soluble solids (2.9 kg) compared with trees infected later (3.1 kg). In 2009, the average soluble solids was 3.3 kg and significantly higher in trees on US-812, US-852, US-897, US-942, and Sour orange (3.4–3.5 kg) compared with Rough lemon (2.9 kg) and US-802 (2.8 kg). Earlier infection with *Las* significantly reduced soluble solids from 3.4 kg to 3.2 kg. In 2010, the average soluble solids was 2.6 kg per box and most solids were obtained for trees on US-812 and US-897 (2.8–2.9 kg). Soluble solids content was not significantly affected by time of infection.

## 4. Discussion

A number of studies have evaluated the performance of different citrus cultivars in response to Huanglongbing, though few reported on the effect of rootstock. This study describes HLB disease development and tree performance in four field trials composed of sweet orange scions on 15 different rootstock selections, which include hybrids of trifoliolate orange and other cultivars, standard for citrus production in Florida. The trials were established in different years and varied in age from two to nine years at the beginning of the study.

In spring 2008, less than three years after the first confirmation of *Las* in Florida, the percentage of infection determined by PCR analysis of leaves ranged from 39% in the oldest trial to 62% in the youngest trial. Though percentages differed between trees on different rootstock varieties, no consistency was observed across the trials, suggesting that infection with *Las* occurred in a manner associated with insect dispersal rather than with rootstock type. By spring of 2009, all trees in the trials were identified as PCR-positive for *Las*. Apparently, despite the initial lower disease incidence observed in the oldest trial, the amount of time for the groves to become completely infected was not affected by age. This is different from other studies conducted in Florida and in other countries where reduced disease incidences were observed in older citrus groves compared with young plantings of less than three years of age (Gottwald, 2010). Close location and similar

environmental conditions of the trials may have contributed to the similar pattern of disease progression found in the present study.

In each trial, foliar HLB symptoms, which included chlorotic patterns often associated with nutrient-deficiencies as well as the blotchy mottle characteristic for HLB, were generally more pronounced in the fall than in the spring of each year. This was particularly noticeable in the first year of the study. It is unclear whether the milder symptom expression in spring is due to a seasonal dormancy or -inactivity of the bacterium, or due to the shorter time of exposure of the leaves produced during the spring flush to the damaging effects of the pathogen. A seasonal change in temperature appeared to cause a similar oscillation in the severity of HLB symptoms on naturally infected sweet orange trees in South Africa, with better defined symptoms occurring during the cooler months (McClellan and Schwarz, 1970). In addition, graft-transmission of the disease was best during the coolest time of the year (Schwarz, 1970). The negative effect of high temperatures on the African form of liberibacter has long been established (Bové, 2006). In contrast, *Las* does not appear to be negatively affected by high temperatures, which in Florida usually exceed 30 °C during the months of May to September. HLB symptoms were generally more severe in trees infected earlier as opposed to later, particularly in the youngest trial in which symptoms were most severe throughout the study. Symptoms were least severe in the trial containing the oldest trees. That older trees are more resistant to HLB was already reported by Lin (1963) in China. Similarly, Bassanezi and Bassanezi (2008) observed lowest disease severities on older trees in citrus groves in Brazil in the absence of HLB control measures. The most likely cause for these findings is the faster distribution of the pathogen within the smaller canopy of young trees along with higher flushing frequencies which attract large numbers of psyllids. HLB symptom expression varied between rootstocks in some of the trials where lowest ratings were observed in trees on US-897. In previous studies conducted in our laboratory, seedlings of US-897 demonstrated tolerance to *Las* as expressed through a slower increase of bacterial DNA levels in the leaves and much reduced disease symptoms compared with other varieties (Albrecht and Bowman, 2011).

Similar to the observations on leaf symptoms, canopy damage was most severe in the youngest trial and least in the oldest one. During the first year of the study, trees infected with *Las* earlier had significantly greater damage than did later infected trees. Least canopy damage was observed for trees on Volkamer lemon in the youngest trials throughout the study. In the oldest trial, trees on US-802 exhibited less canopy damage than did trees on other rootstocks. That rootstock can affect HLB disease expression was also shown by Cheema et al. (1982). Five out of 23 rough lemon selections investigated in that study, including Volkamer lemon, induced tolerance in bud-inoculated greenhouse-grown sweet orange scion. None of the other rootstocks, which included Carrizo citrange, Cleopatra mandarin, Sour orange, and Swingle citrumelo, were shown to induce any resistance. Nariani (1981) tested the reaction of 'Mosambi' orange on twelve rootstock varieties to HLB in India and found that the pathogen was not transmitted when grafted onto sweet lime (*C. limetta*) and Italian- and

**Table 4**  
Canopy damage of sweet orange trees on different rootstocks from spring 2008 to fall 2009.

Rootstock	Canopy damage rank <sup>a</sup>							
	Spring 2008		Fall 2008		Spring 2009		Fall 2009	
<i>Trial 1 ('Valencia', 1999)</i>								
US-801	2.4	a	2.4	a	2.9	ab	3.1	ab
US-802	1.4	a	1.9	a	2.6	b	2.6	b
US-812	2.5	a	2.8	a	3.9	ab	3.9	a
US-852	2.2	a	2.9	a	4.0	a	4.1	a
US-897	2.2	a	2.3	a	3.3	ab	3.2	ab
US-942	2.4	a	2.5	a	3.4	ab	3.5	ab
Carrizo citrange	1.9	a	2.1	a	3.3	ab	3.2	ab
Rough lemon	2.2	a	2.9	a	3.2	ab	3.4	ab
Sour orange	2.5	a	2.8	a	3.5	ab	4.0	a
Sun Chu Sha	2.1	a	2.7	a	3.1	ab	3.2	ab
Swingle citrumelo	2.8	a	3.0	a	4.0	a	4.0	a
Average	2.2		2.6		3.4		3.5	
Early infected <sup>b</sup>	2.7		3.0		3.7		3.6	
Late infected <sup>c</sup>	2.0		2.3		3.2		3.3	
Time of infection	$P < 0.0025$		$P < 0.0026$		$P < 0.0009$		ns	
Rootstock	ns		ns		$P < 0.0080$		$P < 0.0008$	
<i>Trial 2 ('Early Gold', 2002)</i>								
US-801	2.8	a	3.6	a	3.6	a	4.1	a
US-812	3.2	a	4.0	a	3.4	a	3.9	a
US-897	3.0	a	3.7	a	3.7	a	3.8	a
US-942	3.3	a	4.2	a	3.6	a	3.8	a
Carrizo citrange	3.3	a	4.1	a	3.7	a	4.2	a
Kinkoji	3.2	a	4.1	a	3.7	a	3.9	a
Sun Chu Sha	3.1	a	3.6	a	3.3	a	3.7	a
Volkamer lemon	3.1	a	4.0	a	3.4	a	3.9	a
Average	3.1		3.9		3.6		3.9	
Early infected	3.7		4.1		3.8		4.1	
Late infected	2.6		3.7		3.3		3.7	
Time of infection	$P < 0.0001$		$P < 0.0013$		$P < 0.0001$		$P < 0.0001$	
Rootstock	ns		$P < 0.0185$		ns		ns	
<i>Trial 3 ('Valencia', 2004)</i>								
US-802	2.2	bc	2.5	ab	3.6	abc	3.4	cd
US-812	2.2	bc	2.7	ab	4.1	ab	3.8	abc
US-852	3.6	a	2.9	ab	4.2	a	4.2	a
US-897	2.7	abc	2.9	ab	4.1	ab	3.8	abc
US-942	2.8	abc	3.0	ab	4.0	ab	3.5	abcd
Benton citrange	3.0	abc	3.2	ab	4.2	a	4.1	ab
Cleopatra mandarin	2.7	abc	2.7	ab	3.4	bc	3.5	abcd
Kinkoji	2.4	abc	2.7	ab	3.7	abc	3.7	abcd
Sour orange	2.8	abc	3.0	ab	3.9	abc	3.9	abc
Swingle citrumelo	3.2	abc	3.7	a	4.2	a	4.0	abc
Volkamer lemon	2.1	c	2.4	b	3.1	c	3.0	d
Average	2.7		2.9		3.9		3.7	
Early infected	3.1		3.4		3.9		3.8	
Late infected	2.0		2.0		3.8		3.6	
Time of infection	$P < 0.0001$		$P < 0.0001$		ns		ns	
Rootstock	$P < 0.0006$		$P < 0.0479$		$P < 0.0001$		$P < 0.0001$	
<i>Trial 4 ('Valencia', 2006)</i>								
US-802	3.0	a	4.1	a	4.4	ab	4.9	a
US-812	2.3	a	4.0	a	4.4	ab	4.6	a
US-852	3.3	a	4.5	a	4.8	a	5.0	a
US-897	2.8	a	4.0	a	4.6	ab	4.9	a
US-942	2.6	a	3.9	a	4.4	ab	4.7	a
Benton citrange	2.8	a	4.0	a	4.6	ab	4.8	a
Cleopatra mandarin	2.9	a	3.6	a	4.3	ab	4.7	a
Kinkoji	2.8	a	3.7	a	4.5	ab	4.8	a
Sour orange	2.5	a	3.5	a	4.2	ab	4.6	a
Sun Chu Sha	2.8	a	3.3	a	4.5	ab	4.9	a
Swingle citrumelo	2.8	a	4.0	a	4.6	ab	4.8	a
Volkamer lemon	2.3	a	3.3	a	3.7	b	4.2	a
Average	2.7		3.8		4.4		4.7	
Early infected	3.4		4.1		4.6		4.8	
Late infected	2.3		3.6		4.3		4.7	

Table 4 (Continued)

Rootstock	Canopy damage rank <sup>a</sup>			
	Spring 2008	Fall 2008	Spring 2009	Fall 2009
Time of infection	$P < 0.0001$	$P < 0.0003$	$P < 0.0035$	ns
Rootstock	ns	ns	$P < 0.0474$	ns

<sup>a</sup> Canopy damage was ranked from 1 (full tree canopy and little die-back) to 5 (dieback/defoliation in more than 60% of the tree canopy and/or severe stunting of the tree).

<sup>b</sup> PCR-positive for *Ca. L. asiaticus* by spring 2008.

<sup>c</sup> PCR-positive for *Ca. L. asiaticus* after spring 2008. Significant differences were determined using Kruskal-Wallis ANOVA of ranks with  $P < 0.05$  considered as not significant (ns). Numbers in each column followed by different letters are significantly different according to Dunn's post test ( $P < 0.05$ ).

Table 5

Percentage of scion- and rootstock growth of sweet orange trees on different rootstocks from 2006–2008 and from 2008–2010.

Rootstock	Scion growth <sup>a</sup> (%)				Rootstock growth <sup>a</sup> (%)			
	2006–2008		2008–2010		2006–2008		2008–2010	
<i>Trial 1 ('Valencia', 1999)</i>								
US-801	6.2	a	7.2	a	6.5	a	11.7	a
US-802	17.1	a	6.7	a	14.9	a	11.1	a
US-812	6.8	a	5.0	a	5.9	a	9.6	a
US-852	8.9	a	8.9	a	9.7	a	15.2	a
US-897	8.6	a	9.7	a	10.5	a	10.1	a
US-942	7.9	a	5.1	a	9.8	a	11.9	a
Carrizo citrange	9.1	a	1.6	a	3.3	a	11.5	a
Rough lemon	13.2	a	3.7	a	9.5	a	7.5	a
Sour orange	12.7	a	5.1	a	14.2	a	7.4	a
Sun Chu Sha	9.1	a	6.4	a	9.6	a	8.5	a
Swingle citrumelo	5.8	a	6.2	a	7.3	a	7.6	a
Average	9.6		5.9		9.2		10.2	
Early infected <sup>b</sup>	8.1		5.6		8.4		8.9	
Late infected <sup>c</sup>	10.6		6.2		9.7		11.1	
Time of infection	ns		ns		ns		$P = 0.0309$	
Rootstock	ns		ns		ns		ns	
Rootstock x time of infection	ns		ns		ns		ns	
<i>Trial 2 ('Early Gold', 2002)</i>								
US-801	33.9	bc	12.9	ab	32.7	c	19.3	ab
US-812	34.7	bc	12.6	ab	36.3	abc	16.1	ab
US-897	29.3	c	17.9	a	31.6	c	20.8	a
US-942	33.1	bc	13.5	ab	33.5	bc	16.4	ab
Carrizo citrange	36.3	bc	10.4	b	35.4	abc	12.1	b
Kinkoji	48.7	a	13.0	ab	45.9	a	16.8	ab
Sun Chu Sha	43.5	ab	16.0	ab	43.5	ab	20.0	a
Volkamer lemon	42.8	ab	12.9	ab	39.9	abc	14.5	ab
Average	37.8		13.6		37.3		17.0	
Early infected	34.2		12.9		33.2		14.6	
Late infected	41.3		14.4		41.4		19.3	
Time of infection	$P < 0.0001$		ns		$P < 0.0001$		$P = 0.0003$	
Rootstock	$P < 0.0001$		$P = 0.0353$		$P < 0.0001$		$P = 0.0181$	
Rootstock x time of infection	ns		ns		ns		ns	
<i>Trial 3 ('Valencia', 2004)</i>								
US-802	82.9	ab	36.4	ab	79.7	ab	34.6	a
US-812	92.4	a	36.9	ab	84.3	a	38.3	a
US-852	80.6	ab	23.7	b	79.6	ab	26.0	a
US-897	71.8	ab	33.8	ab	64.8	ab	32.6	a
US-942	74.5	ab	31.8	ab	67.1	ab	32.0	a
Benton citrange	62.6	b	27.2	ab	59.6	b	26.4	a
Cleopatra mandarin	80.8	ab	38.3	a	81.8	a	39.7	a
Kinkoji	74.8	ab	31.2	ab	74.1	ab	29.7	a
Sour orange	75.2	ab	32.6	ab	66.8	ab	29.8	a
Swingle citrumelo	65.9	ab	27.6	ab	65.0	ab	32.2	a
Volkamer lemon	83.7	ab	31.7	ab	82.6	a	28.8	a
Average	76.8		32.0		73.1		31.9	
Early infected	71.4		30.0		68.8		30.5	
Late infected	85.6		35.2		80.1		34.1	
Time of infection	$P = 0.0002$		$P = 0.0207$		$P = 0.0005$		ns	
Rootstock	$P = 0.0067$		$P = 0.0196$		$P = 0.0003$		ns	
Rootstock x time of infection	ns		ns		ns		ns	
<i>Trial 4 ('Valencia', 2006)</i>								
US-802	102.7	bc	30.5	ab	95.6	b	29.1	ab
US-812	124.1	abc	33.4	ab	121.1	ab	31.7	ab
US-852	121.0	abc	21.1	b	118.6	ab	19.4	b
US-897	112.8	abc	31.5	ab	108.6	ab	25.7	ab

Table 5 (Continued)

Rootstock	Scion growth <sup>a</sup> (%)				Rootstock growth <sup>a</sup> (%)			
	2006–2008		2008–2010		2006–2008		2008–2010	
US-942	104.4	bc	33.4	ab	103.1	b	29.7	ab
Benton citrange	125.8	abc	26.0	b	119.3	ab	23.0	ab
Cleopatra mandarin	113.7	abc	34.1	ab	106.5	b	29.9	ab
Kinkoji	90.9	c	34.0	ab	96.6	b	28.2	ab
Sour orange	147.1	a	37.0	ab	148.0	a	25.6	ab
Sun Chu Sha	106.8	bc	34.0	ab	103.2	b	32.1	ab
Swingle citrumelo	102.1	bc	26.3	b	98.3	b	24.8	ab
Volkamer lemon	134.5	ab	53.3	a	123.6	ab	44.7	a
Average	115.5		32.9		111.9		28.7	
Early infected	103.9		32.6		98.9		29.4	
Late infected	124.0		33.1		121.4		28.2	
Time of infection	$P=0.0003$		ns		$P=0.0002$		ns	
Rootstock	$P=0.0093$		$P=0.0064$		$P=0.0462$		$P=0.0191$	
Rootstock x time of infection	ns		ns		ns		$P=0.0281$	

<sup>a</sup> Growth was expressed as percent increase of mean stem diameters.

<sup>b</sup> PCR-positive for *Ca. L. asiaticus* by spring 2008.

<sup>c</sup> PCR-positive for *Ca. L. asiaticus* after spring 2008. Significant differences were determined using ANOVA with  $P < 0.05$  considered as not significant (ns). Numbers in each column followed by different letters are significantly different according to Tukey's honestly significant difference test ( $P < 0.05$ ).

Eureka lemon (*C. limon*). Although the disease was transmitted to 'Mosambi' budded on Lisbon lemon, Bengal citron, Carrizo citrange, Troyer citrange, and trifoliolate orange, HLB symptoms remained mild in these combinations and the use of tolerant rootstocks was suggested as one component of an integrated control program in India. According to Shokrollah et al. (2011), no HLB symptoms were observed in mandarin scion when grafted on *C. grandis* rootstock with *C. hystrix* as an interstock. The mechanisms for the induction of tolerance reported for different rootstock varieties, particularly lemons, are unknown. The fuller tree canopies observed on Volkamer lemon in the youngest trials and on US-802 in the oldest trial may be a result of the tendency of these rootstocks to induce very large and vigorous trees (Bowman, 2007a; Castle et al., 2010). However, trees on rough lemon rootstock, which was only available in trial 1, did not perform superior to the other rootstocks in the trial. In addition to HLB, other factors such as canker, leafminers and recent hurricanes likely affected tree performance in all four trials.

Increase in stem diameter of scion and rootstocks measured in 2008 and 2010 was considerably less in the oldest trial compared with the youngest trial. In the oldest trial, growth was not affected

by rootstock or time of infection, likely a result of the generally reduced growth at this stage of maturity. Earlier infection with *Las* reduced scion- and rootstock growth in the younger trials, though predominantly between 2006 and 2008, when not all trees were yet infected. Though rootstock affected stem growth in this time period, results were not consistent across trials. Between 2008 and 2010, stem growth was considerably reduced in all trials compared with 2006–2008, but most noticeably in the youngest trial. In the latter, most growth was observed for trees on Sour orange and Volkamer lemon. It is unclear how much of this growth reduction is a consequence of HLB, tree age or other factors.

Fruit yield was assessed in trial 1 from 2008 to 2009. Trees on the high vigor-inducing rootstocks US-802 and Carrizo citrange (Bowman, 2007a) produced the highest yields, whereas lowest yields were observed in US-897, which is known for its dwarfing effect on the scion (Bowman, 2007b). In 2009, yields were reduced by nearly 50% compared with 2008. It is believed that this large reduction in yield is mainly due to the detrimental effects of *Las*, which was detected in all trees, and not due to annual fluctuation in yield sometimes observed in healthy citrus trees. Large yield

Table 6

Fruit yield and juice soluble solids per box of 'Valencia' trees on different rootstocks planted in 1999 (trial 1).

Rootstock	Yield (kg/tree)				Soluble solids per box <sup>a</sup>					
	2008		2009		2008		2009		2010	
US-801	34.5	b	20.8	a	3.0	bc	3.2	abc	2.7	ab
US-802	62.9	a	30.1	a	2.9	cd	2.8	c	2.3	de
US-812	31.0	b	18.0	a	3.3	a	3.4	a	2.8	a
US-852	33.5	b	19.6	a	3.1	abc	3.5	a	2.6	abcd
US-897	27.4	b	16.4	a	2.9	bcd	3.4	a	2.9	a
US-942	31.4	b	19.0	a	3.1	abc	3.4	a	2.7	ab
Carrizo citrange	64.4	a	30.7	a	3.1	abc	3.2	abc	2.7	ab
Rough lemon	51.1	ab	27.5	a	2.7	d	2.9	bc	2.3	e
Sour orange	32.4	b	19.2	a	3.0	bcd	3.5	a	2.4	cde
Sun Chu Sha	43.3	ab	25.1	a	3.0	bc	3.3	abc	2.4	cde
Swingle citrumelo	28.6	b	16.7	a	3.2	ab	3.4	ab	2.6	abc
Average	40.1		22.1		3.0		3.3		2.6	
Early infected <sup>b</sup>	33.7		23.8		2.9		3.2		2.6	
Late infected <sup>c</sup>	44.1		21.3		3.1		3.4		2.6	
Time of infection	ns		ns		$P=0.0007$		$P=0.0009$		ns	
Rootstock	$P=0.0037$		ns		$P < 0.0001$		$P < 0.0001$		$P < 0.0001$	
Rootstock x time of infection	ns		ns		ns		ns		ns	

<sup>a</sup> Soluble solids were determined as the product of percentage juice and percentage sugar per box (40.8 kg) of fruit.

<sup>b</sup> PCR-positive for *Ca. L. asiaticus* by spring 2008.

<sup>c</sup> PCR-positive for *Ca. L. asiaticus* after spring 2008. Significant differences were determined using ANOVA with  $P < 0.05$  considered as not significant (ns). Numbers in each column followed by different letters are significantly different according to Tukey's honestly significant difference test ( $P < 0.05$ ).

losses primarily due to early fruit drop or lack of fruit set on affected branches were also observed in sweet orange cultivars affected by HLB in Brazil (Bassanezi et al., 2011).

Juice soluble solids content was assessed in trial 1 from 2008 to 2010. Lowest solids content was generally found in fruit produced on rough lemon and US-802, which is characteristic for these genotypes (Bowman, 2007a; Castle, 1995). Fruit from trees on US-812 and US-897 had highest soluble solids in 2010. Van Vuuren and Moll (1985) reported that 'Valencia' fruit HLB symptoms differed significantly among different rootstock cultivars in South Africa. The strongest HLB symptom expression was observed in trees on *P. trifoliata* as compared to Troyer citrange and Empress mandarin. It was suggested that extension of the flushing period, caused by the dwarfing effect of trifoliolate orange and allowing for prolonged feeding by the insect vector, was reason for these findings. In the current study, HLB leaf symptoms of trees on the dwarfing rootstock US-897 were less severe or not different compared with the other, more vigorous rootstocks. In 2008 and 2009 fruit from earlier infected trees had less soluble solids compared with fruit from later infected trees. In 2010, at which time all trees had been infected for nearly two or more years, average solids content was much reduced compared with the previous years. A study by Bassanezi et al. (2009) found reduced soluble solids in HLB symptomatic fruit compared with normal fruit from different sweet orange cultivars on Rangpur lime in Brazil. Effects were more pronounced in fruit from late season 'Valencia' cultivars. According to Baldwin et al. (2010), the negative effects of *Las* on fruit quality are greater in symptomatic than in asymptomatic fruit from infected trees. In the present study fruit were collected randomly without distinguishing asymptomatic fruit from symptomatic ones.

In summary, none of the rootstock varieties evaluated in this study induced high levels of resistance in sweet orange scion in the early years following infection with *Las*. Trees on all 15 rootstocks were considerably damaged by HLB. However, tree performance was improved and tolerance to HLB was increased by some rootstocks, depending on the trial. Tolerance was generally higher in the oldest trees. Our results suggest that high vigor-inducing rootstocks, particularly Volkamer lemon, may enable younger trees to outlast the damaging effects of the disease. It remains to be seen whether some trifoliolate hybrid rootstock varieties will improve performance of scions in the long-term and in the presence of more aggressive nutritional applications and better management of the vector for HLB.

## Acknowledgments

We thank Kerry Worton and Emily Domagtoy for technical assistance. This research was supported in part by grants from the Florida Citrus Production Research Advisory Council and the Florida Citrus Research and Development Foundation. Mention of a trademark, warranty, proprietary product, or vendor does not imply an approval to the exclusion of other products or vendors that also may be suitable.

## References

- Achor, D.S., Exteberria, E., Wang, N., Folimonova, S.Y., Chung, K.R., Albrigo, L.G., 2010. Sequence of anatomical symptom observations in citrus affected with Huanglongbing disease. *Plant Pathol.* **9**, 56–64.
- Albrecht, U., Bowman, K.D., 2011. Tolerance of the trifoliolate citrus hybrid US-897 (*Citrus reticulata* Blanco × *Poncirus trifoliata* L. Raf.) to Huanglongbing. *HortScience* **46**, 16–22.
- Albrecht, U., Bowman, K.D., 2009. *Candidatus Liberibacter asiaticus* and Huanglongbing effects on citrus seeds and seedlings. *HortScience* **44**, 1967–1973.
- Albrecht, U., Bowman, K.D., 2008. Gene expression in *Citrus sinensis* (L.) Osbeck following infection with the bacterial pathogen *Candidatus Liberibacter asiaticus* causing Huanglongbing in Florida. *Plant Sci.* **175**, 291–306.
- Baldwin, E., Plotto, A., Manthey, J., McCollum, G., Bai, J., Irey, M., Cameron, R., Luzzio, G., 2010. Effect of liberibacter infection (Huanglongbing disease) of citrus on orange fruit physiology and fruit/fruit juice quality: chemical and physical analyses. *J. Agric. Food Chem.* **58**, 1247–1262.
- Bassanezi, R.B., Bassanezi, R.C., 2008. An approach to model the impact of Huanglongbing on citrus yield. In: Proceedings of the International Research Conference on Huanglongbing, Orlando, FL, pp. 301–304.
- Bassanezi, R.B., Montesino, L.H., Stuchi, E.S., 2009. Effects of Huanglongbing on fruit quality of sweet orange cultivars in Brazil. *Eur. J. Plant Pathol.* **125**, 565–572.
- Bassanezi, R.B., Montesino, L.H., Gasparoto, M.C.G., Filho, A.B., Amorim, L., 2011. Yield loss caused by Huanglongbing in different sweet orange cultivars in Sao Paulo. *Eur. J. Plant Pathol.* **130**, 577–586.
- Bové, J.M., 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. *J. Plant Pathol.* **88**, 7–37.
- Bowman, K.D., 2007a. Notice to Fruit Growers and Nurserymen Relative to the Naming and Release of the US-802 Citrus Rootstock. United States Dept. of Agric. Agric. Res. Ser.
- Bowman, K.D., 2007b. Notice to Fruit Growers and Nurserymen Relative to the Naming and Release of the US-897 Citrus Rootstock. United States Dept. of Agric. Agric. Res. Ser.
- Castle, W.S., 2010. A career perspective on citrus rootstocks, their development, and commercialization. *HortScience* **45**, 11–15.
- Castle, W.S., 1995. Rootstock as a fruit quality factor in citrus and deciduous tree crops. *N.Z. J. Crop Hort. Sci.* **23**, 383–394.
- Castle, W.S., Baldwin, J.C., Muraro, R.P., Littell, R., 2010. Performance of 'Valencia' sweet orange trees on 12 rootstocks at two locations and an economic interpretation as a basis for rootstock selection. *HortScience* **45**, 523–533.
- Cheema, S.S., Kapur, S.P., Chohan, J.S., 1982. Evaluation of rough lemon strains and other rootstocks against greening-disease of citrus. *Sci. Hortic.* **18**, 71–75.
- Davis, M.J., Mondal, S.N., Chen, H., Rogers, M.E., Brlansky, R.H., 2008. Co-cultivation of '*Candidatus Liberibacter asiaticus*' with actinobacteria from citrus with Huanglongbing. *Plant Dis.* **92**, 1547–1550.
- Fan, J., Chen, C., Brlansky, R.H., Gmitter Jr., F.G., Li, Z.-G., 2010. Changes in carbohydrate metabolism in *Citrus sinensis* infected with '*Candidatus Liberibacter asiaticus*'. *Plant Pathol.* **59**, 1037–1043.
- Folimonova, S.Y., Robertson, C.J., Garnsey, S.M., Gowda, S., Dawson, W.O., 2009. Examination of the responses of different genotypes of citrus to Huanglongbing (citrus greening) under different conditions. *Phytopathology* **99**, 1346–1354.
- Garnier, M., Bové, J.M., Cronje, C.P.R., Sanders, G.M., Korsten, L., Le Roux, H.F., 2000. Presence of *Candidatus Liberibacter africanus* in the Western Cape province of South Africa. In: da Graça, J.V., Lee, R.F., Yokomi, R.K. (Eds.), Proc. 14th Conf. Int. Organ. Citrus Virologists. IOCV, Riverside, CA, pp. 369–372.
- Gottwald, T.R., 2010. Current epidemiological understanding of citrus Huanglongbing. *Annu. Rev. Phytopathol.* **48**, 199–139.
- Halbert, S.E., 2005. The discovery of Huanglongbing in Florida. In: Proceedings of the 2nd International Citrus Canker and Huanglongbing Research Workshop, Florida Citrus Mutual, Orlando, FL, p. H-3.
- Halbert, S.E., Manjunath, K.L., 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus. A literature review and assessment of risk in Florida. *FL Entomol.* **87**, 330–353.
- Hartung, J.S., Halbert, S.E., Pelz-Stelinski, K., Brlansky, R.H., Chen, C., Gmitter, F., 2010. Lack of evidence for transmission of '*Candidatus Liberibacter asiaticus*' through citrus seed taken from affected fruit. *Plant Dis.* **94**, 1200–1205.
- Kim, J.S., Sagaram, U.S., Burns, J.K., Li, J.-L., Wang, N., 2009. Response of sweet orange (*Citrus sinensis*) to '*Candidatus Liberibacter asiaticus*' infection: microscopy and microarray analyses. *Phytopathology* **99**, 50–57.
- Lin, K.H., 1956. Yellow shoot of citrus. Symptomatology. Investigations in the cause of Huanglongbing. Natural transmission and spread. General conclusion. *Acta Phytopathol. Sin.* **2**, 1–42.
- Lin, K.H., 1963. Further studies on citrus yellow shoot. *Acta Phytophylact. Sin.* **2**, 243–251.
- Lou, B.H., Zhou, C.Y., Zhao, X.Y., Li, Z.G., Xu, M., Liu, J.X., Zhou, Y., Tang, K.Z., 2010. Primary study on species and intraspecific differentiations of HLB pathogens in eight provinces of China. In: Deng, X., Xu, J., Lin, S., Guan, R. (Eds.), Proceedings of the International Society of Citriculture, vol. 2. China Agricultural Press, Wuhan, China, p. 1195.
- McClellan, A.P.D., Schwarz, R.E., 1970. Greening or blotchy-mottle disease of citrus. *Phytophylactica* **2**, 177–194.
- Miyakawa, T., 1980. Experimentally-induced symptoms and host range of citrus likubin (greening disease). *Ann. Phytopath. Soc. Japan* **46**, 224–230.
- Miyakawa, T., Zhao, X.Y., 1990. Citrus host range of greening disease. In: Aubert, B., Tontyaporn, S., Buangsuwon, D. (Eds.), Proceedings of the 4th International Asia Pacific Conference on Citrus Rehabilitation. FAO-UNDP, pp. 118–121.
- Nariani, T.K., 1981. Integrated approach to control citrus greening disease in India. *Proc. Int. Soc. Citricult.* **1**, 471–472.
- Saunt, J., 2000. Citrus Varieties of the World. An Illustrated Guide, second edition. Sinclair International Ltd., Norwich, England.
- Schneider, H., 1968. Anatomy of greening-diseased sweet orange shoots. *Phytopathology* **58**, 262–266.
- Schwarz, R.E., 1970. Seasonal graft-transmissibility and quantification of gentsoyl glucoside marker of citrus greening in the bark of infected trees. *Phytophylactica* **2**, 115–120.
- Sechler, A., Schuenzel, E.L., Cooke, P., Donnua, S., Thavechai, N., Postnikova, E., Stone, A.L., Schneider, W.L., Damsteeg, V.D., Schaad, N.W., 2009. Cultivation of '*Candidatus Liberibacter asiaticus*', '*Ca. L. africanus*', and '*Ca. L. americanus*' associated with Huanglongbing. *Phytopathology* **99**, 480–486.

- Shokrollah, H., Abdullah, T.L., Sijam, K., Abdullah, S.N.A., Abdullah, N.A.P., 2009. Differential reaction of citrus species in Malaysia to Huanglongbing (HLB) disease using grafting method. *Am. J. Agric. Biol. Sci.* 4, 32–38.
- Shokrollah, H., Abdullah, T.L., Sijam, K., Abdullah, S.N.A., 2011. Potential use of selected citrus rootstocks and interstocks against HLB disease in Malaysia. *Crop Prot.* 30, 521–525.
- Teixeira, D.C., Ayres, A.J., Kitajima, E.W., Tanaka, F.A.O., Danet, J.L., Jagoueix-Eveillard, S., Saillard, C., Bové, J.M., 2005. First report of a Huanglongbing-like disease of citrus in Sao Paulo State, Brazil, and association of a new liberibacter species, *Candidatus Liberibacter americanus*, with the disease. *Plant Dis.* 89, 107.
- Teixeira, D.C., Wulff, N.A., Martins, E.C., Kitajima, E.W., Bassanezi, R., Ayres, A.J., Eveillard, S., Saillard, C., Bové, J.M., 2008. A phytoplasma closely related to the pigeon pea witches'-broom phytoplasma (16Sr IX) is associated with citrus Huanglongbing symptoms in the state of São Paulo, Brazil. *Phytopathology* 98, 977–984.
- Van Vuuren, S.P., Moll, J.N., 1985. Influence of the rootstocks on greening fruit symptoms. *Citrus Subtrop. Fruit J.* 612, 7, 10.