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Performance of 'Valencia' orange (*Citrus sinensis* [L.] Osbeck) on 17 rootstocks in a trial severely affected by huanglongbing

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ABSTRACT

'Valencia' orange (*Citrus sinensis* [L.] Osbeck) was grown on 17 rootstocks through seven years of age and the first four harvest seasons in a central Florida field trial severely affected by huanglongbing (HLB) disease. All trees in the trial had HLB symptoms and were shown by PCR to be infected with *Candidatus* Liberibacter asiaticus (Las). Large differences were noted between rootstocks for many metrics examined, including yield, fruit quality, and tree size. Highest yields in the trial were on US-942 rootstock, which was significantly more productive than trees on the common commercial rootstocks Carrizo, Kuharske, Cleopatra, and Kinkoji. Other new hybrid rootstocks also performed well in this trial strongly affected by HLB, including the rootstock US-1516, which had the second highest cumulative yield, best tree health rating, and lowest number of trees lost due to HLB damage. Comparison of tree performance in this trial with a similar trial conducted prior to the HLB epidemic, allows us to estimate that the disease resulted in a 33% reduction in yield and 21% reduction in tree growth through seven years of age. Use of a tolerant rootstock is suggested as an effective means of ameliorating crop losses to HLB.

1. Introduction

Profitability of citrus production is strongly influenced by the rootstock used in the planting. Carefully conducted field trials to compare citrus rootstock performance have demonstrated that there can be more than a six-fold difference in yield between the best and worst rootstocks (Wutscher and Bowman, 1999), with rootstocks also exhibiting clear significant effects on tree survival, fruit quality, tree size, and tolerance to numerous biotic and abiotic threats (Barry et al., 2004; Bowman et al., 2003; Castle et al., 2011; Wutscher and Hill, 1995). Previous reports of rootstock field trials affected by huanglongbing (Albrecht et al., 2012; Nariani, 1981; Cheema et al., 1982; Van Vuuren and Moll, 1985) have yielded confusing and sometimes contradictory results, in part, because of erratic disease spread and irregular development of symptoms. We describe details of performance for 17 rootstocks in a field trial with 'Valencia' sweet orange established in a commercial grove in central Florida, which became 100% infected with *Candidatus* Liberibacter asiaticus (Las), the believed causal agent for huanglongbing (HLB) in Florida, by the time the planting reached 7 years of age. The results suggest significant differences between rootstocks in their influence on tree tolerance to HLB.

2. Materials and methods

2.1. Tree propagation

In this trial, seven unreleased U.S. Department of Agriculture hybrid rootstocks were compared with ten commercially available rootstocks (Table 1). Seven of the commercially available rootstocks used (Carrizo, Swingle, US-802, US-812, US-852, US-897, and US-942) were developed and released previously by the USDA breeding program. Seeds of all the selections were grown in Brite Leaf citrus nursery (Lake Panasoffkee, Florida), budded with the scion 'Valencia' clone 55-1 in 2007, and prepared for field planting. The 'Valencia' clone used was obtained from Florida Department of Agriculture and Consumer Services, where it was tested and found free of citrus tristeza virus (CTV), Las, and other known pathogens using conventional indexing methods (Kesinger, 2007).

2.2. Field planting

The budded trees were transplanted into the trial in June 2008 at a commercial field site in Polk County, Florida, owned by Wheeler Citrus, at a spacing of 4.4 m × 7.6 m. The experiment included 21

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Table 1
Rootstocks tested with parentage or species.

Rootstock	Parentage/species
Carrizo	*Citrus sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.
Cleopatra	*C. reticulata* Blanco
Kinkoji	*C. obovoidea* Takahashi
Kuharske	*C. sinensis* × *P. trifoliata*
Swingle	*C. paradisi* Macf. × *P. trifoliata*
US-801	*C. reticulata* 'Changsha' × *P. trifoliata* 'English Small'
US-802	*C. grandis* (L.) Osbeck 'Siamese' × *P. trifoliata* 'Gotha Road'
US-809	*C. reticulata* 'Changsha' × *P. trifoliata* 'English Large'
US-812	*C. reticulata* 'Sunki' × *P. trifoliata* 'Benecke'
US-827	*C. reticulata* hybrid 'Rangpur' × *P. trifoliata*
US-852	*C. reticulata* 'Changsha' × *P. trifoliata* 'English Large'
US-896	*C. reticulata* 'Cleopatra' × *P. trifoliata* 'Rubidoux'
US-897	*C. reticulata* 'Cleopatra' × *P. trifoliata* 'Flying Dragon'
US-942	*C. reticulata* 'Sunki' × *P. trifoliata* 'Flying Dragon'
US-1503	*C. grandis* 'African' × *P. trifoliata* 'Flying Dragon'
US-1516	*C. grandis* 'African' × *P. trifoliata* 'Flying Dragon'
US-1524	*C. grandis* 'African' × *P. trifoliata* 'Flying Dragon'

'Valencia' trees on each of the 17 rootstocks, planted in a randomized complete block design, in ten adjacent rows about 200 m long. Border trees with the same scion were planted on each end of the rows and in the two adjacent rows. Soil was Candler sand, with good natural drainage, and a gentle slope. Irrigation in the block was by under-tree microjets.

2.3. Field plot management

The rootstock trial planting was adjacent to a commercial block of trees with 'Valencia' scion, and management care of the trial block was the same as the contiguous commercial block. General care of the block was as follows. During the bearing years, fertilizer was applied by broadcast spreader in three equal applications of 12-2-16 with Mg and Zn, at a rate of 72 kg N per acre per year. Weeds were controlled by 3 applications/year of glyphosate (Roundup; Monsanto, St. Louis MO) and Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea; Loveland Products, Loveland, CO] in a 2.4 m band within the row, from 2008 through 2012. From 2012 to 2015, weed control was by application of glyphosate within the row as needed. Row middles were developed with bahia grass cover and were managed with periodic mowing. Chemical pest control was applied five times per year using a speed sprayer. The first application was in the spring at 2/3 petal fall and contained copper hydroxide and spirotetramat (Movento; Bayer CropScience, Research Triangle Park, NC). A second treatment, containing oil, copper hydroxide, and abamectin (Agri-Mek; Syngenta Crop Protection, Greensboro, NC), was applied when the May flush reached full expansion. A mixture of thiamethoxam and abamectin (Agri-Flex; Syngenta Crop Protection) was applied three times per year to combat insects and mites.

2.4. Tree yield and fruit quality

Yields for groups of three trees (seven replicates of three trees for each rootstock) were measured during harvest in April of each year, 2012–2015, by a cooperative effort of the commercial harvesting crew and USDA research staff. Yield/tree and cumulative yield/tree was calculated based on the trees that remained (surviving trees) in the planting at the time of each season's harvest, except where it is indicated that numbers are based on the number of planted trees (pt). Samples of 24-fruit for quality analysis were collected from each replicate of three trees just before harvest each year. Fruit samples were juiced using an FMC Multifruit Fresh N'squeeze juicer, model POS1 (JBT FoodTech Citrus Systems, Lakeland, FL), and juice quality was analyzed using standard laboratory methods.

2.5. Tree size

Tree size measurements of scion trunk cross sectional area (TCA) 5 cm above the graft union, canopy height, and canopy spread were taken in July 2015 using tape measure, height pole, and caliper. Canopy volume was calculated by the formula (diameter^2 × height)/4 (Wutscher and Hill, 1995). Yield efficiency was calculated as the ratio of fruit yield per surviving tree in 2015 to canopy volume in July 2015.

2.6. Detection of Las and CTV

For PCR detection of Las in trees, eight leaves were collected from each plant in July 2015, two from each cardinal direction using only mature leaves from the latest flush at a height of 1.5–2.0 m above the ground. Petioles and parts of the midrib were severed from leaves and ground in liquid nitrogen with a mortar and pestle. One-hundred 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, but omitting RNA removal to allow for detection of citrus tristeza virus (CTV) and Las in the same sample. For detection of Las, real-time PCR assays were performed using primers HLBas and HLBr and probe HLBp developed by Li et al. (2006). For normalization, all samples were assayed using primers COXf and COXr and probe COXp (Li et al., 2006). Amplifications were performed over 40 cycles using an ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA) and the QuantiTect Probe PCR Kit (Qiagen) according to the manufacturer's instructions. All reactions were carried out in a 20 μl reaction volume using 5 μl of extracted DNA/RNA. Plants were considered PCR-positive when normalized Ct_{LAS}-values were ≤ 33.

For quantification of Las, Ct_{LAS}-values were converted to copy numbers of Las genomes based on a standard curve created in our laboratory: $y = 12.34–0.32x$, where y = log of Las copy number and x = normalized Ct_{LAS}-value. Ct-values not determined after 40 cycles were assigned a value of 41. Since three copies of the 16s rDNA gene are present in the Las genome (Duan et al., 2009), copy numbers were divided by three and data were expressed as numbers of Las genomes per milligram of plant tissue.

For detection of CTV, real-time PCR assays were performed using primers UTR1 and UTR2 and probe 181T (Bertolini et al., 2008). Amplifications were carried out in an AB7500 real-time PCR system using the QuantiTect Probe RT-PCR Kit (Qiagen) according to the manufacturer's instructions and using 2 μl of extracted DNA/RNA in a 20 μl reaction volume.

2.7. Foliar disease symptoms

Trees were examined for chlorosis, blotchy mottle, green islands, or other foliar abnormalities presumably associated with HLB in April 2015, and scored on a scale of 0–5 (0 = no foliar disease symptoms; 1 = <10% foliar disease symptoms, dense canopy; 2 = 10–25% foliar disease symptoms, dense canopy; 3 = 25–50% foliar disease symptoms, mostly dense canopy/few areas with open canopy/few rabbit ears; 4 = 50–75% foliar disease symptoms, open canopy/rabbit ears abundant; 5 = >75% foliar disease symptoms, canopy very open/rabbit ears very abundant).

2.8. Statistical analyses

Data were tested by analysis of variance using Statistica ver 10.0 (StatSoft, Tulsa, Okla.) and comparison of the means was by Duncan multiple range test at $P < 0.05$. Foliar disease symptom ratings were tested by Kruskal–Wallis ANOVA by Ranks.

3. Results

3.1. Fruit yield

Fruit yield was measured during the harvest seasons 2012–2015, and across all rootstocks averaged 43 kg per tree per season. There were significant differences among the rootstocks in fruit yield during each of the four harvest seasons, 2012–2015, with US-942 having the highest production each of the first three seasons and highest cumulative yield (Table 2), and averaging 57 kg of fruit per tree per season. Other rootstocks in the trial that are commonly used in Florida, including Swingle, US-802, Carrizo, Kuharske, US-812, and Cleopatra, were intermediate in yield per tree, ranging from 39 to 45 kg/tree/season. The two rootstocks with the lowest yield in the trial were Kinkoji and US-801, both averaging 32 kg fruit/tree/season. Among the other rootstocks that are not commercially available, US-1516, US-1503, US-1524, and US-896 yielded particularly well, ranging from 48–53 kg/tree/season.

3.2. Fruit quality

Fruit quality parameters were measured during each of the seasons 2012–2015. Analysis of variance for main effects of harvest year and rootstock were highly significant for each of the fruit quality parameters averaged over the four years (Table 3). Although harvest year by rootstock interactions for fruit weight, total acidity and juice color were significant for each parameter, the interaction effect was a minimal component of the variance. Largest mean fruit weight (222 g) was obtained with Kuharske, and smallest (173 g, 180 g and 184 g) with US-801, US-897 and US-896, respectively, while most of the other hybrids produced fruit not significantly different in weight from Kuharske.

The greatest difference in percent juice was between fruit produced on US-809 (57.5%) compared with US-802 (54.7%) and US-1516 (54.8%), which were not significantly different. There was considerable similarity in percent juice among the additional rootstocks. Total soluble solids (TSS) ranged from a low of 9.15% for fruit produced on Cleopatra to a high of 9.97% for fruit produced on US-896. Fruit produced on Swingle had TSS of 9.19% which was not significantly different from Cleopatra. Most of the hybrid rootstocks produced fruit with TSS lower than, but not significantly different from, US-896. Total acidity was lowest (0.71%) in fruit produced on Cleopatra and highest (0.88%) in fruit produced on US-801. Highest TSS:Acid ratios (13.2:1 and 13.1:1) were in fruit produced on Kuharske and Cleopatra, and lowest (11.5:1) on US-897 and US-801. Although main effects of rootstock on juice color were statistically significant, the range in color number was only from 38.2 to 38.6, indicating no practical difference among the rootstocks.

Although all fruit quality parameters differed significantly among the harvest years, clear trends were only seen for fruit weight and TSS. Mean fruit weights decreased from a maximum of 228 g in 2012 to a minimum of 194 g in 2015, representing a 15% decrease over the four years in which data were collected. The lowest TSS (9.1%) were recorded in 2012 (Table 3) and highest (10.3%) in 2013. The low TSS in 2012 may have been a function of tree age, as it is known that citrus fruit TSS is typically lower from less mature than from more mature trees. Between 2013 and 2015 mean TSS dropped from 10.3 to 9.4, a 15% decrease.

3.3. Tree size and yield efficiency

Scion trunk cross sectional area (TCA), canopy height, canopy spread, and canopy volume were influenced significantly by rootstock when measured at 7 years of age (Table 4). Trees on US-802 rootstock were the largest by all measures of size except TCA, where trees on Cleopatra rootstock were largest. Trees on US-897 rootstock were the smallest by all measures of size. Trees on US-801, the rootstock that appeared most strongly affected by HLB, were intermediate in size, and the canopy volume of trees on this rootstock were not significantly different from those on Cleopatra, Carrizo, Kuharske, Kinkoji, or Swingle. Yield efficiency values ranged from 3.73 kg/m^3 for US-801 to 11.31 kg/m^3 for Swingle.

Fig. 1. Photo from the trial taken just before harvest, showing a tree with mild (left) and severe (right) symptoms of HLB.

3.4. CTV and Las infection and HLB effects

The average percent of trees that were CTV positive across all rootstocks was 27.4%. The proportion of trees that were positive by rootstock ranged from 5.6–47.1%. Of the 357 trees originally planted in the trial, 52 trees were removed by the grower between 2009 and harvest in 2015 because of poor health (primarily symptoms of HLB). All of the 305 trees that remained in the trial in July 2015 from the original planting tested positive by PCR for Las, and 304 of the trees showed visible leaf symptoms of HLB (Fig. 1). Mean Ct_{Las} values for the remaining trees differed significantly by rootstock (Table 5), with trees on US-801 having the lowest Ct_{Las} value (and calculated highest Las copy number), and US-897 and US-896 having the highest Ct_{Las} (and calculated lowest Las copy number). Although there were significant differences between Ct_{Las} values for rootstocks, trees on all rootstocks had a large amount of the bacteria present in tissues sampled. Tree removal in the trial by the grower/cooperator was based on visual symptoms of HLB not PCR results, but it can be noted that US-801 also had the largest number of trees removed by the cooperator by July 2015. Although statistical comparisons of the visual tree rating for HLB symptoms in April 2015 were not significant ($P > 0.05$), it can be observed that surviving trees on US-801 had the highest mean value for HLB symptoms at that time.

4. Discussion

This study investigated the performance of 'Valencia' sweet orange on 17 rootstocks in a commercial trial conducted in Polk County, Florida (USA), between 2008 and 2015. The trial was strongly affected by HLB, as indicated by nearly 100% of the trees showing symptoms of HLB and testing positive for Las infection by PCR by 7 years of age. As has been noted previously (Bowman and McCollum, 2015), HLB appears to rarely cause field tree death in Florida, but typically causes a decline in tree health that results in a sparse canopy and little marketable fruit. The resulting unhealthy trees are usually abandoned or removed by citrus growers. In this

Table 2
Yield of 'Valencia' trees on 17 rootstocks in a Polk county trial.

Rootstock	Yield 2012 (kg/tree)	Yield 2013 (kg/tree)	Yield 2014 (kg/tree)	Yield 2015 (kg/tree)	Yield 2012–15 (kg/tree)
US-942	43 a	67 a	66 a	52 abc	227 a
US-1516	32 bc	60 abc	54 abc	65 a	211 ab
US-896	36 ab	64 ab	61 ab	44 abc	205 abc
US-1503	29 bcd	47 cde	60 ab	61 ab	197 abc
US-1524	27 bcd	48 cde	56 abc	63 ab	193 abc
Swingle	27 bcd	47 cde	50 a–d	56 abc	180 a–d
US-802	27 bcd	59 bcd	47 a–d	43 abc	176 bcd
Carrizo	25 cd	49 b–e	48 a–d	45 abc	168 bcd
US-852	31 bc	49 b–e	41 a–d	45 abc	167 bcd
Kuharske	32 bc	45 cde	47 a–d	42 a–d	166 bcd
US-809	28 bcd	50 b–e	40 bcd	46 abc	164 bcd
US-812	35 abc	47 cde	35 cd	44 abc	162 bcd
Cleopatra	16 e	34 e	51 a–d	57 abc	158 cd
US-827	27 bcd	42 de	34 cd	38 bcd	141 d
US-897	34 abc	38 e	32 cd	33 cd	137 d
Kinkoji	19 de	39 e	38 bcd	34 cd	130 d
US-801	36 abc	47 cde	27 d	19 d	128 d

Mean separations for significant ANOVA within columns were by Duncan's multiple range test at $P < 0.05$.

Table 3
Fruit quality of 'Valencia' trees on 17 rootstocks in a Polk county trial assessed for the years 2012–2015.

	Fruit wt (g)	Juice (%)	TSS (%)	Acid (%)	Ratio	Juice color (CN)
Rootstock						
US-896	184 d	57.2 ab	9.97 a	0.84 a–c	12.0 bc	38.4 ab
US-801	173 d	55.6 b–d	9.97 ab	0.88 a	11.5 c	38.3 ab
US-897	180 d	56.5 a–c	9.81 a–c	0.87 ab	11.5 c	38.2 b
Carrizo	205 bc	55.7 b–d	9.74 a–d	0.80 c–e	12.3 a–c	38.4 ab
US-942	204 bc	56.9 ab	9.73 a–d	0.81 b–d	12.0 bd	38.3 ab
US-852	217 a–c	56.0 a–d	9.72 a–d	0.79 c–e	12.5 ab	38.2 b
Kuharske	222 a	55.9 b–d	9.69 a–e	0.74 ef	13.2 a	38.6 a
Kinkoji	202 c	56.7 a–c	9.64 a–e	0.78 c–e	12.4 a-c	38.2 b
US-809	202 c	57.5 a	9.61 a–e	0.78 c–e	12.4 a-c	38.3 ab
US-1503	212 a–c	56.0 a–d	9.60 a–e	0.82 b–d	11.9 bc	38.3 ab
US-1524	210 a–c	55.3 cd	9.59 a–e	0.78 c–e	12.4 a-c	38.4 ab
US-802	217 a–c	54.7 d	9.55 a–e	0.81 b–d	11.9 bc	38.3 ab
US-827	215 a–c	56.2 a–d	9.50 a–e	0.84 a–c	11.6 c	38.2 b
US-1516	212 a–c	54.8 d	9.41 b–e	0.76 de	12.5 ab	38.6 a
US-812	210 a–c	56.2 a–d	9.35 c–e	0.77 de	12.4 a–c	38.4 ab
Swingle	213 a–c	56.3 a–d	9.19 de	0.74 ef	12.5 ab	38.6 a
Cleopatra	219 ab	56.2 a–d	9.15 e	0.71 f	13.1 a	38.5 ab
Year						
2012	228 a	57.4 a	9.1 d	0.75 c	12.3 b	38.5 a
2013	217 bt	55.3 c	10.3 a	0.81 b	12.8 a	38.5 a
2014	183 d	56.3 b	9.6 b	0.87 a	11.2 c	–
2015	194 c	55.4 c	9.4 c	0.75 c	12.7 a	38.1 b
Factor (df)						
Replicate (6)	1.87***	0.002*	2.27**	0.035***	3.5 ns	0.44*
Year (3)	29.1***	0.011***	28.15***	0.406***	64.2***	4.34***
Rootstock (16)	3.11***	0.002***	1.48**	0.057***	6.8***	0.36***
Year × Rootstock (48)	0.62***	0.001 ns	0.8 ns	0.012*	2.0 ns	0.28*
Error (400)	0.35	0.001	0.8	0.009	2.1	0.18
Total (473)						

Fruit quality data presented for individual rootstocks are averaged over years 2012–2015. Mean separations for significant ANOVA within columns were by Duncan's multiple range test at $P < 0.05$. *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

trial, the grower removed about 15% of the originally planted trees by 7 years of age because of poor performance and pronounced visible symptoms of HLB.

Although this field trial was not originally focused on testing rootstocks for tolerance of huanglongbing disease in Florida, the speed and extent to which it became infected by the pathogen Las created a good opportunity to evaluate rootstock effect on tree tolerance to the disease. Trees in the trial also became infected with CTV, with 27.4% of trees testing positive for the virus at seven years of age, but the isolate of CTV found in the area of the trial is known to have no significant effect on growth of trees on the rootstocks included in this trial (Bowman and Garnsey, 2001). Rootstock was seen to have a significant effect on most of the important traits measured in the trial, including fruit production, fruit quality, tree size, and Ct_{Las} of the scion. The results from this trial provided much better definition of rootstock effects on tree performance under pressure from HLB than our previous report on rootstock field trials with HLB (Albrecht et al., 2012). We believe this is primarily because the previous report relied strongly upon tree growth and HLB symptom scores rather than multi-year yield and fruit quality data, as well as because those trials used fewer replications and trees per rootstock that were more subject to effects from uneven disease spread. Natural spread of Las into field plantings can be erratic and non-uniform over time and area, and this can fatally

Table 4
Tree size of 'Valencia' trees on 17 rootstocks in a Polk county trial measured in 2015.

Rootstock	ScionTCA (cm^2)	Canopy height(m)	Canopy diameter (m)	Canopy volume (m^3)	Yield efficiency (kg/m^3)
US-802	97.3 a	2.84 a	3.23 a	7.44 a	5.78
US-1503	89.2 ab	2.71 a–c	3.05 a–c	6.47 ab	9.43
US-812	81.0 b–d	2.67 a–d	3.06 a–c	6.41 a–c	6.86
US-1516	86.7 a–c	2.63 a–e	3.07 ab	6.28 a–d	10.35
US-942	80.6 b–d	2.67 a–d	3.03 a–d	6.23 a–e	8.35
US-827	77.4 b–e	2.75 ab	3.00 b–d	6.20 b–e	6.13
US-1524	80.8 b-d	2.66 a–d	3.01 b–d	6.08 b–e	10.36
Cleopatra	98.6 a	2.44 de	2.96 b–d	5.53 b–f	10.31
Carrizo	79.3 b–d	2.46 c–e	2.86 b–f	5.35 b–f	8.41
Kuharske	89.2 ab	2.56 b-e	2.87 b–f	5.35 b–f	7.85
Kinkoji	86.7 a–c	2.49 c-e	2.86 b–f	5.13 c–g	6.63
US-801	71.8 c–f	2.53 b–e	2.85 b–f	5.10 d–g	3.73
US-896	64.0 e–g	2.39 e	2.90 b–e	5.09 d–g	8.64
US-852	76.9 b-e	2.49 c–e	2.83 c–f	5.00 d–g	9.00
Swingle	65.6 d–g	2.42 de	2.81 d–f	4.95 e–g	11.31
US-809	60.2 fg	2.40 ef	2.71 ef	4.47 fg	10.29
US-897	53.7 g	2.17 f	2.68 f	3.92 g	8.42

Mean separations for significant ANOVA within columns were by Duncan's multiple range test at $P < 0.05$

Table 5
Infection with Las and effects of HLB for 'Valencia' trees on 17 rootstocks in a Polk county trial.

Rootstock	Mean Ct Las	Las copy number/mg plant tissue	Trees removed for HLB (%)	Surviving tree health rating	Kruskal–Wallis mean rank[a]
US-896	27.8 a	301	10	3.3	151
US-897	27.7 ab	374	19	3.1	128
Kuharske	27.2 a–c	513	0	3.3	144
US-1516	27.0 a–c	428	0	3.1	117
US-942	26.9 a–c	641	14	3.4	151
US-809	26.9 a–d	590	19	3.4	158
Swingle	26.8 a–d	515	10	3.3	140
Carrizo	26.7 b–d	713	19	3.1	143
US-852	26.6 b–d	728	19	3.5	159
US-1524	26.6 cd	761	14	3.3	157
US-1503	26.6 cd	639	5	3.2	139
Kinkoji	26.5 cd	642	14	3.4	161
US-827	26.4 cd	617	29	3.6	189
Cleopatra	26.4 cd	748	14	3.7	185
US-812	26.3 cd	765	5	3.2	135
US-802	26.2 cd	798	5	3.7	182
US-801	25.8 d	1,050	52	3.8	192

Mean separations for significant ANOVA within columns were by Duncan's.
multiple range test at $P < 0.05$.
[a] Kruskal–Wallis ANOVA test was applied to tree health rating. $P = 0.3668$.

compromise field trials with insufficient statistical replications. Differences in results between that previous report and this study can also be attributed, in part, to the different location in which the present study was conducted. In this trial, as in many other locations in Florida now, rootstock tolerance to HLB has a dominant effect on overall tree performance. However, it is important to recognize that performance of citrus rootstocks in the field is affected by many different abiotic and biotic factors, and that relative rootstock field performance will often vary considerably between trials in different locations.

Estimation of the quantitative effects of HLB on 'Valencia' tree performance can be obtained by comparison of this study to another rootstock trial with the same scion conducted under very similar conditions between 1991 and 1998, before the HLB epidemic in Florida (Bowman and Rouse, 2006; Wutscher and Bowman, 1999). Although other factors may play a part in the differences in yield, tree loss, and tree size between the two trials, we believe that the predominant factor in these differences is Las infection and the effect of HLB. A comparison of yield, tree loss, and tree size (TCA) for the five rootstocks in common for the two trials (Table 6) shows a substantially reduced performance in the trial affected by HLB. Yield reduction in the HLB affected trial through the first 7 years (first four harvests) ranged by rootstock from 22–49%, with an average reduction of 33%. Reduction in tree size, as measured by TCA at 7 years of age, ranged from 2 to 34%, with an average reduction in tree size of 21%. Although we have not attempted to calculate the economic impact of HLB in this study, it is clear that the 33% yield loss will have a large effect on profitability for the commercial grower.

Studies of rootstock genotype response to Las infection without grafting and under greenhouse conditions have proven valuable for understanding of Las infection and HLB disease development (Albrecht and Bowman, 2011,2012), but they appear insufficient to make good predictions about ultimate field performance of grafted trees infected with Las. The results of this study indicate the strong potential to identify significant and commercially meaningful differences between rootstocks in field tolerance to HLB by the use of carefully designed and conducted field trials. Among the commercially available rootstocks, US-942 (Bowman, 2010) appeared to have a clear advantage for commercial use under the conditions of this trial, by yielding the most fruit and having good fruit quality. In the comparison with a second trial without HLB (Table 6), US-942 also appeared to be one of the rootstocks least affected by HLB in terms of cropping and tree growth. As with other rootstocks previously identified as exhibiting improved field tolerance to HLB (Bowman and McCollum, 2015b), we believe the reaction of US-942 to Las infection is best described as tolerance, since sweet orange trees on this rootstock still become infected with

Table 6
Comparison of performance of 'Valencia' on five rootstocks in this trial, with a similar trial conducted in Polk county before the HLB epidemic (Wutscher and Bowman, 1999).

Rootstock	Results from trial not affected by HLB[a]		Results from this trial, affected by HLB		Comparison of the two trials	
	Kg yield per tree	Trees removed (%)	Kg yield per tree st[b] (pt)	Trees removed (%)	Percent yield loss per st (pt) due to HLB	Percent tree TCA reduction due to HLB
US-942	291	4	227 (215)	14	22 (26)	2
Swingle	240	0	180 (169)	10	25 (30)	19
Carrizo	216	0	168 (163)	19	22 (25)	31
US-812	306	0	162 (157)	5	47 (49)	19
US-827	235	0	141 (132)	29	40 (44)	34

[a] Results from Wutscher and Bowman, 1999.
[b] Cumulative yield of fruit (kg) per surviving tree (st) or per planted tree (pt), through the first four harvest seasons for 'Valencia' on these rootstocks in this trial.

Las, maintain relatively high titers of the bacteria, and show leaf symptoms.

Among rootstocks included in the trial that are not commercially available, US-801 deserves special note because it exhibited the lowest yields, smallest fruit size, highest fruit acid, highest proportion of trees removed, lowest Ct_{Las} values, highest Las copy number, and the highest symptom rating of surviving trees. The exceptionally poor performance of US-801 rootstock in this trial suggests an exceptional sensitivity to HLB, since its performance was good in other trials before the introduction of Las to Florida (Wutscher and Hill, 1995). Among other rootstocks in the trial that are not commercially used in Florida, US-1516 and US-896 deserve particular attention because of a combination of high yield and good fruit quality. Trees on these two rootstocks differed significantly in size (TCA), with US-1516 making a larger tree than US-896. It can also be noted that trees on US-1516 had the lowest percent of tree loss through seven years (0%), and lowest HLB symptom score (3.1) of any rootstock in the trial. Since the use of rootstocks which maintain higher yields and good fruit quality despite Las infection appears an effective way of ameliorating the effects of HLB disease, US-1516 was released by USDA in October 2015 (Bowman and McCollum, 2015a).

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