Influence of Citrus Source and Test Genotypes on Inoculations with *Candidatus Liberibacter asiaticus*

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Abstract. Assessments of the resistance of citrus germplasm to huanglongbing (HLB) can be expedited by inoculating plants under laboratory or greenhouse settings with the HLB bacterium, *Candidatus Liberibacter asiaticus* (CLAs). Consistent rapid screening is critical to efficiently assess disease resistance among plant materials; however, a number of factors may govern the efficacy of such inoculations. Despite the rapidity at which HLB can spread in a grove, it often takes 8 to 10 months for high levels of CLAs and HLB symptoms to develop even in highly susceptible sweet orange. Therefore, two experiments were conducted to assess factors that might influence efficiency in screening for HLB resistance. In one experiment, three test citrus genotypes ('Kuharske', previously shown to be HLB resistant; rough lemon, previously shown to be HLB tolerant; and 'Valencia', HLB susceptible) were bud grafted using CLAs-infected buds from four different source genotypes. All bud source genotypes had similar levels of CLAs titer, but citron, rough lemon, and Volkamer lemon were hypothesized to be better bud inoculum sources as they are more tolerant of HLB than 'Valencia'. Among the three test genotypes over all sources of infected buds, inoculations of 'Kuharske' resulted in lower CLAs titers and fewer HLB symptoms than inoculations of rough lemon or 'Valencia'. Inoculations of rough lemon resulted in higher CLAs titers and more pronounced HLB symptoms when it was inoculated using infected buds from rough lemon or 'Valencia'. Grafting 'Valencia' with infected buds from Volkamer lemon resulted in less disease than when 'Valencia' was grafted with infected citron, rough lemon, or 'Valencia' buds. Overall, these results suggest that the source of CLAs-infected buds used to graft-inoculate some genotypes will influence disease development. Trunk cross-sectional area increase for the year following infection was 3× higher in 'Kuharske' and rough lemon, compared with 'Valencia'. 'Kuharske' had very low levels of CLAs (30 CLAs/μg DNA), whereas 'Valencia' (43,000 CLAs/μg DNA) and rough lemon (6700 CLAs/μg DNA) had relatively high levels. As an alternative to graft-inoculating plants with CLAs-infected buds, plants can be subjected to infestations of CLAs-infected Asian citrus psyllid (ACP) as occurs naturally. Of interest is if transmission rates of CLAs and the development of HLB in a genotype are greater when the ACP have been feeding on the same host genotype. An experiment was therefore conducted to assess transmission of CLAs by ACP reared on CLAs-infected rough lemon to five different genotypes ('Carrizo', 'Flame' grapefruit, rough lemon, 'Temple', and 'Valencia'). These assessments were made using a detached leaf assay recognized as a faster method of gauging transmission rates of CLAs than using whole plants. Higher percentages of ACP died when they were transferred from infected rough lemon to healthy 'Carrizo', and lower percentages died when they were transferred to rough lemon or 'Flame'. However, CLAs transmission by infected ACP occurred to at least some leaves of each genotype in each of the five different assays, with 70% or more leaves of each genotype becoming infected in at least one assay. Over all assays, there was relatively little variation among genotypes in the percentage of leaves becoming CLAs infected and in the titer of CLAs developing in infected leaves. However, there were relatively large differences in transmission rates among individual assays unrelated to differences among test genotypes. Because of the rapidity of the detached leaf assay, efforts are merited to improve consistency of this inoculation method.

Huanglongbing disease is considered the greatest threat to the U.S. citrus industry (Gottwald, 2010). In the United States, HLB is associated with CLAs and its insect

vector, *Diaphorina citri*, the ACP. Following initial infection, symptoms may take many months to become evident. As the disease progresses, trees become less vigorous, with

thinning canopies and abnormal leaves contributing to yield loss and poor fruit quality (Bové, 2006; Gottwald, 2010). Since HLB was first confirmed in Florida in 2005, it has been found in all 32 citrus producing counties in the state as well as in dooryard citrus in noncitrus producing counties (Halbert et al., 2008). It is becoming increasingly widespread in Texas, and several HLB-affected dooryard trees have now been identified in California (CDFA, 2015).

Management of HLB in Florida focuses on insecticide applications for vector control in combination with a series of production practices intended to reduce stress in infected trees. These practices have markedly increased production costs per hectare, while productivity in infected Florida orchards is reported to show an estimated average reduction of 41% compared with healthy orchards (Singer and Useche, 2015). A more sustainable approach for the management of HLB would be the cultivation of citrus cultivars which are unaffected, or less affected, by the disease rather than more susceptible types (Gottwald, 2010).

Several studies indicate differences in HLB susceptibility among citrus cultivars and/or species, which may be sufficient to be economically significant (Stover and McCollum, 2011; Stover et al., 2015a, 2016). However, despite the rapid and devastating spread of HLB throughout Florida, it is remarkably difficult to quickly and efficiently screen for relative resistance and tolerance to HLB. Following graft-inoculation or exposure of germplasm to CLAs-infected ACP, it often takes 8–10 months to observe a high level of CLAs infection even in highly susceptible sweet orange. In discussions with many scientists studying HLB, several anecdotal observations suggested opportunities to improve inoculation efficiency.

Many graft inoculations are made using buds from highly HLB-susceptible sweet orange which may have compromised health and reduced ability to transfer CLAs. Therefore, this study investigates whether a graft-inoculum source showing more tolerance to HLB would enhance CLAs transfer and facilitate identifying differences in HLB susceptibility.

Some researchers reported that transition of ACP from one genotype to another appeared to compromise ACP health and consequent transfer of CLAs. Therefore, we also examined whether ACP, reared on a specific test genotype and infected with CLAs, could transfer CLAs to the same test genotype at a higher rate than to other genotypes. This was evaluated using a detached leaf inoculation test that was reported to be a faster approach to assessing CLAs transmission rates than using whole plants (Ammar et al., 2013).

Materials and Methods

All experimental trees were maintained in a greenhouse at the U.S. Horticultural

Table 1. Ten trees of each of three citrus genotypes ('Kuharske' citrange, rough lemon, and 'Valencia') were double bud inoculated 20 Mar. 2012 with highly HLB symptomatic citron, rough lemon, 'Valencia', or Volkamer lemon. In May 2012, trees were rebudded with the same source genotype if both prior buds were dead. For each bud, the leaf petiole was removed and assessed for CLas titer. Development of CLas titer was followed over time in inoculated trees and development of distinctive HLB mottling recorded. Petioles and leaf midribs from mature leaves, from flushes with some lignification on stems, were assessed for CLas titer using qPCR.

Source of variation	Rebudding needed (%)	CLas titer petiole from bud (Ct)	Percent leaves with HLB mottle				Petiolar CLas titer (Ct)		
			Nov. 2012	Jan. 2013	Mar. 2013	May 2013	Jan. 2013	Mar. 2013	May 2013
Test genotype grafted									
Kuharske	0 b ^z	23.4 b ^y	0.8 b	0.0 b	0.1 b	0.0 b	41.0 a	38.1 a	42.4 a
Rough lemon	33 a	26.5 a	12.4 a	11.9 a	8.6 a	6.3 a	33.0 b	30.8 b	30.0 b
Valencia	10 b	24.8 ab	7.3 a	10.9 a	7.1 a	5.7 a	30.8 b	27.5 b	27.2 b
Source of infected bud ^x									
Citron	35 a	26.5 ab	5.5 b	3.0 c	1.5 c	3.1 bc	34.6 b	32.7 b	28.4 a
Rough lemon	5 b	24.0 b	17.0 a	24.5 a	17.3 a	13.0 a	26.6 a	24.1 a	27.8 a
Valencia	20 ab	26.9 a	15.8 a	16.0 b	11.3 b	6.6 b	29.2 a	25.8 a	26.1 a
Volkamer lemon	25 ab	25.3 ab	0.8 b	1.6 c	1.1 c	1.1 c	37.7 b	34.4 b	32.6 a
Test genotype grafted/source of infected bud									
Kuharske/Citron	0 d	23.0 c	0.0 d	0.0 d	0.0 e	0.0 d	42.2 ab	38.3 ab	40.2 ab
Kuharske/Roughlem	0 d	22.4 c	0.0 d	0.0 d	0.0 e	0.0 d	43.6 a	36.8 b	43.9 a
Kuharske/Valencia	0 d	22.5 c	0.0 d	0.0 d	0.5 de	0.0 d	38.3 b–d	36.5 b	45.0 a
Kuharske/Volklem	0 d	26.2 ab	3.0 cd	0.0 d	0.0 E	0.0 d	39.8 a–c	40.9 a	40.4 ab
Roughlem/Citron	60 a	30.5 a	0.0 d	0.0 d	0.0 e	0.2 cd	37.9 cd	35.7 bc	34.0 bc
Roughlem/Roughlem	10 cd	23.9 bc	21.0 a	24.0 a	17.5 ab	14.0 ab	27.6 e	24.8 ef	24.9 de
Roughlem/Valencia	10 cd	27.0 ab	27.5 a	22.5 ab	17.0 ab	11.0 ab	27.9 e	25.3 f	27.4 c–e
Roughlem/Volklem	50 ab	24.5 bc	1.0 d	1.0 d	0.0 e	0.0 d	38.8 a–c	37.4 ab	34.0 bc
Valencia/Citron	10 cd	22.6 c	11.0 b	6.0 cd	3.0 cd	6.0 b	31.3 de	29.8 de	22.1 e
Valencia/Roughlem	0 d	24.1 bc	13.0 a	25.0 a	17.0 a	12.0 a	25.6 e	23.3 f	31.5 cd
Valencia/Valencia	30 bc	26.7 ab	4.0 bc	9.5 bc	5.5 bc	2.1 c	30.4 e	26.2 d–f	24.8 de
Valencia/Volklem	0 d	26.2 ab	0.6 d	2.2 cd	2.2 c–e	2.2 dc	36.5 b–d	31.1 cd	31.3 cd

CLas = *Candidatus Liberibacter asiaticus*; Ct = cycle threshold; HLB = huanglongbing; qPCR = quantitative polymerase chain reaction.

^zWithin a column and section, means followed by the same letter are not significantly different at $P \leq 0.05$.

^yCt corresponds to genomes of CLas/μg DNA as follows: 22 = 1,800,000, 27 = 76,000, 32 = 3300, 37 = 140, and 42 = 6.

^xInoculation source data exclude the 'Kuharske' test trees since response was negligible in this genotype.

Research Laboratory (USHRL) in Fort Pierce, FL.

Graft inoculations

Six trees, each of 'Valencia' (*Citrus sinensis*, budded onto US-812, *Citrus reticulata* × *Poncirus trifoliata*), rough lemon (*Citrus jambhiri*, nucellar seedlings), citron (*Citrus medica*, cultivar Mhac Nao from rooted cuttings), and Volkamer lemon (*Citrus volkamariana*, nucellar seedlings) were graft inoculated on 20 Oct. 2010 with CLas using infected 'Valencia' budwood at three buds per tree, and these trees are hereafter referred to as source trees, because they were then used as sources of budwood for further graft inoculations. Rough lemon was shown to be more tolerant of HLB than 'Valencia', with sustained phloem transport even in heavily symptomatic leaves (Fan et al., 2012). Our observations indicate that Volkamer lemon and citron are also more HLB tolerant than sweet orange, and exceed rough

lemon in maintaining relatively full canopies in HLB-infected trees, whereas 'Valencia' has substantial leaf loss and develops very thin canopies (Miles et al., 2016). The original inoculum of CLas was from an infected 'Lisbon' lemon (*Citrus limon*) from a commercial grove near Fort Pierce, FL (Albrecht and Bowman, 2008); it has been used extensively in experiments, with infected trees sustained through serial graft transmission.

These source trees developed distinctive HLB mottling and CLas titer, and provided material for graft inoculations of three test tree genotypes: 'Valencia' (budded on rough lemon), 'Kuharske' (*C. sinensis* × *P. trifoliata*, from apomictic seed), and rough lemon (from apomictic seed). On 20 Mar. 2012, the three test genotypes were graft inoculated using buds from each of the four source genotypes. Two buds from the same source genotype were grafted onto each of 10 individuals of each test genotype, resulting in 10 trees each of 12 combinations (4 source genotypes × 3 target genotypes). Source buds with attached leaves were randomly assigned to test trees. Buds were cut from branches clearly showing blotchy mottle symptoms, which is a distinctive symptom of HLB, and buds with attached leaves were randomly assigned to test trees. Two source buds of the same genotype were used for each test tree. The petioles and midribs of leaves from each bud were frozen for quantitative polymerase chain reaction (qPCR) of CLas titer with complete accounting of which leaf corresponded to which bud. On 31 May 2012, any tree where both inoculum buds were dead received an additional two buds

from the same initial inoculum source. At the time of bud-inoculation, test trees had a diameter of 4 to 8 mm and a height of 0.5 to 0.9 m. All trunk diameter measurements were made at 5 cm above the soil surface, which was just above the graft union on the budded trees.

After budding, data were collected seven times between June 2012 and June 2013 on trunk diameter and percentage of leaves showing distinct blotchy mottle symptoms on each test tree. Mature leaves from flushes with some lignification on stems were collected on the same dates and were assessed for CLas titer using qPCR.

Detached leaf assays with ACP

The experiments were conducted using the detached leaf assay described in Ammar et al. (2013). All ACP were adults from a colony maintained in a USHRL insectary on rough lemon which had been graft infected with the same CLas 'Lisbon' lemon source described above. CLas titer was determined on a subsample of colony ACP on the same date the leaves were exposed, using the method described in Hall et al. (2016). A single leaf was considered to be an experimental unit and ACP transfer was made to all leaves on the same day. Ten ACP were placed on 10 mature leaves each from 'Flame' grapefruit (*Citrus paradisi*), 'Temple' tangor (*C. reticulata* × *C. sinensis*), 'Valencia' sweet orange, 'Carrizo' (*C. sinensis* × *P. trifoliata*), and rough lemon. The experiment was conducted five times from Mar. 2013 to Feb. 2014, using leaf samples from trees with no recent insecticide

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applications. Sample leaves were checked to verify that no ACP mortality occurred within 24 h following placement. ACP mortality on the experiment leaves was also assessed at the end of the 1-week experimental feeding period. The leaves were incubated for an additional week and then CLas titer was determined based on qPCR of the petiole/midrib of each leaf.

Determination of CLas titer in leaves

All leaf samples were stored at -20°C until processed. For each tree, the lower 2 cm from the leaf petiole base into the midrib were macerated in a Fast Prep 24 (MP Biomedicals, Santa Ana, CA) with DNA extracted from 200 mg of macerated tissue using CTAB extraction followed by column clean up and concentration (Genesee Scientific, San Diego, CA). Each sample was assayed for CLas and citrus dehydrin via qPCR using an ABI 7500 thermocycler (Applied Biosystems, Foster City, CA). Each 25 μL qPCR reaction was conducted using 100 ng of template DNA (standardized using Nanodrop, Thermo Fisher Scientific, Waltham, MA) with primers specific to 16S rDNA for CLas detection (Las Long): USHRL-CL1-L 5'-CTTACCAGCCCTTGACATGTATAGG-3' and USHRL-CL1-R 5'-TCCCTATAAAGTACCCAACATCTAGGTAAA-3' or Citrus dehydrin (CD-L 5'-TGAGTAGAGCCGAGTGTTG-3' and CD-R 5'-AAACTTCACCGATCCACCAG-3'). Citrus dehydrin was used as reference control to verify DNA quality and PCR amplification. The master mix consisted of Promega (Madison, WI) GoTaq qPCR with BRYT Green and 0.2 μM of each primer using cycle parameters of 2 min at 95°C start, 50 cycles of 15 s at 95°C , 20 s at 60°C , and 20 s at 72°C , with a melt curve analysis (to verify product identity). From cycle threshold (Ct) values, numbers of CLas genomes were calculated per μg DNA analyzed, based on previously published laboratory analyses (Stover et al., 2015b).

Statistical analyses

All data including qPCR results (analyzed as Ct) were assessed using the nonparametric Kruskal–Wallis test (SAS Institute). Where CLas was found to be nondetectable, a Ct value of 45 (which is less than one copy of target DNA sequence per sample analyzed) was used in the analyses to provide a reasonable value for calculations. To assess the relationship between growth and CLas titer, regressions were run between the Ct in June 2013 and the percentage trunk cross-sectional area increase over the preceding year (Excel; Microsoft, Redmond, WA). For the inoculativity study, we analyzed data which included all leaves and also using only data from leaves with detectable CLas.

Results and Discussion

Graft inoculations

Incidence of both buds on a test tree dying from initial inoculation. Among the budwood

test genotypes, rough lemon trees displayed death of both buds from initial inoculations in 33% of trees vs. 0% in 'Kuharske' and 10% in 'Valencia' (Table 1). 'Kuharske' showed no need for rebudding with any source budwood and later showed virtually no mottling or CLas, therefore the main effects of inoculation source genotypes were compared excluding the 'Kuharske' data. As a source genotype the rough lemon budwood had the fewest failures, with only 5% of trees requiring rebudding vs. 20% to 35% for the others (Table 1), although only the citron with 35% failures was different at $P = 0.05$. When assessing individual means for statistically significant interactions, citron and Volkamer lemon source budwood had the highest failure rate in rough lemon test trees, whereas 'Valencia' source budwood had a higher failure than rough lemon or Volkamer lemon for 'Valencia' test trees.

CLas titer in petioles/midribs. At the first budding date, there was remarkable

uniformity in CLas titer between the different bud source genotypes and the inoculum provided to each test tree was also virtually identical (data not shown). But at the date of rebudding, the CLas titer was lower (higher Ct), and therefore, where rebudding was necessary, both test and source genotypes showed lower mean titers for leaf petioles associated with inoculum buds.

'Kuharske' is a selection of 'Carrizo' which has been previously shown to have few HLB symptoms and develop low pathogen titer (Folimonova et al., 2009), and the 'Kuharske' test trees had very low CLas titer throughout the 15 months of the experiment. However, rough lemon and 'Valencia' had moderate titers by Jan. 2013 which continued through the last assessment and were not statistically different from each other. Therefore, data presented on CLas titer as influenced by main effect of inoculum source excluded 'Kuharske' test trees. Averaged across all trees, with the exception of

Fig. 1. Trunk diameter averaged across 10 trees each of three citrus genotypes ('Kuharske' citrange, rough lemon, and 'Valencia') double bud inoculated 20 Mar. 2012 with highly huanglongbing symptomatic citron, rough lemon, 'Valencia', or Volkamer lemon. On each date, trunk diameters are not significantly different at the $P = 0.05$ level if marked by the same letter. Following May 2012, there was no statistically significant effect of bud inoculum source on growth. There was no significant interaction between inoculated genotype and bud source. (A) Effect of bud inoculum source genotype (10 trees $\times$ 3 inoculated genotypes = 30 trees with each budwood source). (B) Effect of test genotype (10 trees $\times$ 4 budwood sources = 40 trees of each genotype).

'Kuharske', trees inoculated with rough lemon and 'Valencia' buds had significantly higher CLas titers than those inoculated with buds from citron or Volkamer lemon in Jan. and Mar. 2013, but by May 2013 there were no statistical differences in titer as influenced by source genotype.

At 10 months after inoculation (Jan. 2013), the petiolar CLas titers between test trees were statistically different between the resistant 'Kuharske' vs. the susceptible 'Valencia' and tolerant rough lemon, for all inoculum source genotypes except Volkamer lemon. Petiolar CLas titers between test trees were statistically different between 'Kuharske' vs. 'Valencia' and rough lemon for the ensuing 4 months when either 'Valencia' or rough lemon was used as the inoculum source.

There were a few notable interactions between inoculum source and test genotypes. At 10 months after inoculation (Jan. 2013), rough lemon source budwood produced lower CLas titers in 'Kuharske' than did 'Valencia' or citron, although all 'Kuharske' titers were very low. In 'Valencia' test trees inoculated with citron source budwood, titers were never statistically different from 'Valencia' inoculated with 'Valencia' budwood and citron produced higher titer than did rough lemon by May 2013.

Development of distinctive HLB mottle. Negligible mottle was observed before Nov. 2012. 'Kuharske' test trees never displayed more than slight mottle symptoms. Neither citron nor Volkamer lemon source budwood conferred high levels of mottle symptoms to rough lemon or 'Valencia' test genotypes, despite having similar levels of CLas in source budwood. Marked budwood source genotypes by test genotype interactions were observed in 'Valencia' and rough lemon. When assessing test tree by source tree

interactions, rough lemon and 'Valencia' source budwood were usually equally effective in inducing CLas titer and HLB blotchy mottle except that rough lemon source budwood produced a greater percentage of HLB mottle than did 'Valencia' budwood in the 'Valencia' test genotype.

Tree growth. In May 2012 (Fig. 1), test trees inoculated with rough lemon were slightly larger in trunk diameter than trees inoculated with citron (7.3 vs. 6.9 mm). After that time, main effect of budwood source genotype averaged across test trees was never statistically significant. However, there was a marked difference in tree growth between the test genotypes (Fig. 1). The 'Valencia' trees were significantly larger than the 'Kuharske' trees at the start of the experiment, but grew considerably less than the other test genotypes. Most notably, rough lemon, which displayed greater mottle than 'Valencia' test trees and had similar high CLas titers, increased in trunk diameter much the same as the HLB-resistant 'Kuharske' with very low CLas titer. Calculated from trunk diameter, the trunk cross-sectional area increase from May 2012 to May 2013 was 3.51-fold in 'Kuharske', 3.56-fold in rough lemon, and 1.22-fold in 'Valencia'. Although rough lemon is known to produce a very vigorous tree, healthy 'Valencia' on rough lemon also grows vigorously. This difference in rough lemon and 'Valencia' growth while displaying similar levels of CLas and HLB symptoms is indicative of the tolerance to HLB displayed by rough lemon (Fan et al., 2012) and some related genotypes (Miles et al., 2016). Regressions were conducted for each test genotype, assessing the relationship between the June 2013 Ct for CLas and the trunk cross-sectional area increase over the preceding year, using only

trees with detectable CLas. Much lower r^2 for rough lemon ($r^2 = 0.106$, $P = 0.064$) compared with 'Valencia' ($r^2 = 0.383$, $P < 0.0001$) provides further evidence of some HLB tolerance in rough lemon. The same regression analysis in 'Kuharske' provided an $r^2 = 0.012$ and $P = 0.77$.

Detached leaf assays with ACP

The incidence of CLas-infected ACP and associated titer levels are known to be variable even in colonies raised on highly infected plants (Hall et al., 2016). In our assays, the source ACP populations ranged from 30% to 80% infected when using a threshold of Ct ≤ 36 (Table 2). For ACP with Ct ≤ 36 , mean Ct ranged from 27.9 to 30.8. At the end of each assay, we did not assay individual ACP from each leaf, thus the inoculum load that each leaf was subjected to is not known. Some mortality of ACP occurred during the 1-week infestations (Table 2). This is consistent with the results of Ammar et al. (2013), who noted that some mortality of ACP adults occurred in their assays with detached leaves. In our assays, percentage mortality of ACP was significantly higher among those infesting 'Carrizo' as opposed to the four other genotypes during one assay, and was in the highest mortality statistical grouping in all five assays. In a study by Richardson and Hall (2013), ACP maintained on some citranges had shorter life spans, although 'Carrizo' was not among those studied. Regardless of ACP mortality, CLas transmission occurred to at least some leaves of each genotype in each of five different assays with at least 40% of leaves of each genotype becoming infected in two assays (Table 2). The highest percentages of leaves becoming infected occurred during the June assay—higher transmission rates in this particular assay as opposed to others were probably a consequence of higher

Table 2. Detached leaf assay with ACP. At each date, 10 detached leaves each from 'Flame' grapefruit, 'Temple' tangor, 'Valencia' sweet orange, 'Carrizo' rootstock, and rough lemon rootstock, were subjected to CLas + ACP feeding on detached leaves for 1 week. All ACP were from a colony maintained in the USHRL insectary, with same original CLas source used in the graft inoculation experiments in this report. CLas titer was determined on a sample of source ACP on the date leaves were exposed. The experiment was conducted four times from Mar. 2013 to Feb. 2014, using leaf material from trees with no recent insecticide applications, and verified to cause no ACP mortality following 24 h of ACP feeding. ACP mortality was assessed at the end of the 1-week experimental feeding period. CLas titer was determined for the petiole/midrib of each leaf, with means across all leaves, and only those with detectable CLas (some amplification of target rDNA).

	Psyllid mortality (%)					CLas titer all leaves (Ct)				
	28 Mar.	17 Apr.	13 June	21 Aug.	7 Feb.	28 Mar.	17 Apr.	13 June	21 Aug.	7 Feb.
Carrizo	18.8 ab ^z	19.0 a	18.4 a	77.8 a	54.4 a	39.1 a ^y	36.0 bc	31.9 b	37.9 a	40.9 a
Flame	21.2 ab	4.9 b	17.0 a	6.9 c	26.8 bc	38.8 a	38.8 ab	37.6 a	39.0 a	38.8 ab
Rough lemon	10.6 b	15.7 a	13.2 a	25.2 b	31.1 a-c	37.1 a	34.6 c	36.5 ab	41.9 a	36.2 b
Temple	27.2 ab	23.5 a	13.9 a	22.7 b	43.3 ab	35.0 a	40.7 a	33.8 ab	43.5 a	40.0 ab
Valencia	36.6 a	17.6 ab	23.0 a	49.0 b	20.6 c	31.0 a	41.4 a	33.5 ab	43.5 a	40.8 ab
	Leaves with detectable CLas (%)					CLas titer only leaves with detectable CLas (Ct)				
	28 Mar.	17 Apr.	13 June	21 Aug.	7 Feb.	28 Mar.	17 Apr.	13 June	21 Aug.	7 Feb.
Carrizo	50 a	70 ab	100 a	40 a	50 a	33.2 a	32.2 b	31.9 a	27.3 b	38.1 b
Flame	50 a	60 ab	70 ab	40 a	60 a	32.6 a	34.6 a	34.4 a	30.0 ab	34.7 b
Rough lemon	50 a	80 a	60 b	20 a	80 a	29.2 a	32.1 b	30.9 a	29.4 ab	34.0 b
Temple	70 a	40 ab	90 ab	10 a	50 a	30.7 a	34.3 a	32.6 a	30.0 ab	36.6 ab
Valencia	60 a	30 b	90 ab	10 a	40 a	21.7 b	33.1 ab	33.5 a	37.7 a	34.5 b
	Source ACP with Ct <36 (%)					CLas titer for source ACP with Ct <36 (Ct)				
	28 Mar.	17 Apr.	13 June	21 Aug.	7 Feb.	28 Mar.	17 Apr.	13 June	21 Aug.	7 Feb.
	30	50	80	50	60	30.8	29.2	27.9	29.8	28.8

ACP = Asian citrus psyllid; CLas = *Candidatus Liberibacter asiaticus*; Ct = cycle threshold; USHRL = U.S. Horticultural Research Laboratory.

^zWithin a column and section, means followed by the same letter are not significantly different at $P \leq 0.05$.

^yCt corresponds to genomes of CLas/ μ g DNA as follows: 22 = 1,800,000, 27 = 76,000, 32 = 3300, 37 = 140, and 42 = 6.

Table 3. Summary comparing traits of CLas-inoculum budwood source genotypes in an experiment in which three citrus genotypes ('Kuharske' citrange, rough lemon, and 'Valencia') were double bud inoculated with highly HLB symptomatic citron, rough lemon, 'Valencia', or Volkamer lemon. Higher number of "+" symbols for source genotype in each category indicates more favorable characteristics as an inoculum source to impart HLB symptoms and CLas titer.

Inoculum budsource genotypes	CLas titer in buds used in inoculation ^z	Shoot growth in infected trees used as budsource ^y	Budtake without need for rebudding ^x	Production of mottle symptoms in recipient plants ^x	Development of CLas titer in recipient plants	
					At 10–12 mo. after inoculation ^x	At 14 mo. after inoculation ^x
Citron	+++	+++	+	+	+	++
Rough lemon	+++	+++	+++	+++	+++	++
Valencia	+++	+	++	++	+++	++
Volkamer lemon	+++	+++	++	+	+	++

CLas = *Candidatus Liberibacter asiaticus*; HLB = huanglongbing.

^zData in Table 1 show some differences in average CLas titer, but these numbers include material used in rebudding when titers were lower. For initial bud inoculations, all source genotypes had similar titers.

^yData not shown.

^xExtracted from data in Table 1.

numbers of CLas infected ACP per leaf in combination with higher titers of CLas per ACP. Over all genotypes and assays, an average of 55% of leaves developed detectable levels of CLas and the Ct value associated with infected leaves averaged 32.4 (Table 2). Over all assays, there was relatively little variation among genotypes in the percentage of leaves becoming CLas infected (range from 46% to 62%) or in the titer of CLas developing in infected leaves (Ct range from 31.1 to 32.8). However, there were larger differences in transmission rates among assays with respect to percentages of leaves becoming infected (range 24% to 82%) and average Ct values of infected leaves (range 29.5 to 35.6). The results generally indicated similar transmission rates of CLas occurred to each of the five genotypes by ACP reared on infected rough lemon. Based on research reports by Hall et al. (2016), detached leaf assays conducted to assess CLas resistance may provide more consistent inoculation if fully expanded flush leaves are used rather than mature leaves, as used here.

Conclusions

Experiments were conducted to assess factors which might permit more rapid screening for HLB resistance. Consistency of CLas titer and effectiveness in infecting test trees was evaluated using several source genotypes for graft inoculations. Rough lemon and 'Valencia' were much better sources of graft inoculum than citron or Volkamer lemon, even though all four source genotypes had similar CLas titer levels. Rough lemon as source trees for inoculum produced much more budwood than 'Valencia' and had the lowest incidence of bud mortality in test trees, making rough lemon an especially good source of bud inoculum in these studies (Table 3).

Trunk cross-sectional area increase was 3× higher in 'Kuharske' and rough lemon, compared with 'Valencia': 'Kuharske' had

very low levels of CLas (30/μg DNA), whereas 'Valencia' (43,000/μg DNA) and rough lemon (6700/μg DNA) had relatively high levels. This is indicative of the tolerance to HLB displayed by rough lemon.

In detached leaf assays, where ACP from infected rough lemon were used to inoculate five different genotypes, no major differences were observed among test genotypes in transmission rates of CLas. From a time standpoint, results of the detached leaf assays could be obtained within 2 to 3 weeks as compared with the 11 to 15 months required for our graft inoculations of whole plants. However, this would only be useful in assessing resistance and not tolerance.

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