Long-Term Field Evaluation Reveals Huanglongbing Resistance in *Citrus* Relatives

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Abstract

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Citrus huanglongbing (HLB) is a destructive disease with no known cure. To identify sources of HLB resistance in the subfamily Aurantioideae to which citrus belongs, we conducted a six-year field trial under natural disease challenge conditions in an HLB endemic region. The study included 65 *Citrus* accessions and 33 accessions belonging to 20 other closely related genera. For each accession, eight seedling trees were evaluated. Based on quantitative polymerase chain reaction analysis of the pathogen titers and disease symptoms, eight disease-response categories were identified. We report two immune, six resistant, and 14 tolerant accessions. Resistance and tolerance observed in different accessions may

be attributed to a multitude of factors, including psyllid colonization ability, absence of pathogen multiplication, transient replication of the bacterium, lack of pathogen establishment in the plant, delayed infection, or recovery from infection. Most citrus cultivars were considered susceptible: 15 citrons, lemons, and limes retained leaves in spite of the disease status. Resistance and high levels of field tolerance were observed in many non-citrus genera. Disease resistance/tolerance was observed in Australian citrus relative genera *Eremocitrus* and *Microcitrus*, which are sexually compatible with citrus and may be useful in future breeding trials to impart HLB resistance to cultivated citrus.

Citrus huanglongbing (HLB) or greening is a formidable disease that has caused enormous economic loss to many citrus growing regions of the world (Bové 2006; da Graça and Korsten, 2004; Halbert and Manjunath 2004). HLB has been in India and China for over a hundred years (Husain and Nath 1927; Chen 1943). The disease was first reported in the Western hemisphere about 12 years ago (Coletta-Filho et al. 2004; Halbert 2005). HLB is associated with an unculturable α -proteobacterium, '*Candidatus Liberibacter*' and is transmitted by two psyllid vectors, *Diaphorina citri* Kuwayama and *Trioza erytreae* (del Guercio). At least three species of the bacterium, '*Ca. L. asiaticus*', '*Ca. L. africanus*', and '*Ca. L. americanus*', are known to be associated with HLB. Of the three species, '*Ca. L. asiaticus*' is the most virulent and widely distributed worldwide.

In the 10 years since its introduction, the Florida citrus industry has lost over 50% of its citrus and production is decreasing at an alarming rate (Gottwald 2010; USDA National Agricultural Statistics Service 2015). In California, the vector *Diaphorina citri* (Asian citrus psyllid [ACP]) was first reported near the border with Mexico, near San Diego in 2008. Based on the Florida experience as well as recently developed research information, California was proactive and established several regulatory and operational guidelines to prevent, eradicate, or delay the introduction and spread of both ACP and HLB. Consequently, the rate of vector and disease spread has been significantly reduced in California compared with Florida. However, neither the preventive efforts nor the management practices currently being followed in HLB endemic regions of the world are sufficient for the long-term survival of an economically feasible and environmentally affordable citrus industry. Development of resistance to

HLB, either through traditional breeding, by transgenic approaches, or both appears to be the best long-term solution for survival of the citrus industry.

HLB-resistant citrus cultivars are not reported in the literature. Most of the early work on cultivar assessment was based on symptomatology. Interpretation of the disease evaluation data were often difficult because of similarities to other disease symptoms and poor cultural practices resulting in nutritional deficiencies that could be mistaken for HLB. In addition, a reliable laboratory diagnostic method for the agent associated with HLB was not available before 2005. Many rutaceous plants carrying HLB-associated bacteria may not develop characteristic HLB symptoms, such as blotchy leaf mottle, loss of foliage, and lopsided fruit. Disease evaluation studies conducted in recent times utilize nucleic acid-based detection of the HLB-associated bacterium. Detection of the pathogen at a single time point may not be adequate to understand the HLB disease status of the taxa. Many citrus relatives appear to support the replication of Liberibacters for a short period of time before suppressing them completely. Indian wood apple (*Limonia acidissima*; syn. *Feronia acidissima*) is reported to be a transient host of '*Ca. L. asiaticus*' (Hung et al. 2000; Koizumi et al. 1996). *Murraya* species are reported to have very low titers of '*Ca. L. asiaticus*' and are reported as possible transient hosts of Liberibacter spp. (Beattie et al. 2012; Walter et al. 2012a).

Inconsistency in published reports regarding disease response of citrus cultivars to HLB can be confusing; the variability observed may be due to host genotypes, geographical location, cultural practices, or differences in pathogenicity of diverse liberibacter isolates. Pummelos from many Asian countries were reported to be HLB resistant, but recent studies from the same geographic regions report that pummelos are susceptible (Ahmad et al. 2008; Shokrollah et al. 2009). HLB-resistant pummelos are still present in Vietnam and India (M. L. Keremane, *personal observation*). *Murraya paniculata* has been reported as HLB resistant from Taiwan (Hung et al. 2000, 2001) but as susceptible to both '*Ca. L. asiaticus*' and '*Ca. L. americanus*' from Brazil (Lopes et al. 2010).

Citrus has been cultivated for many centuries. Both clonal propagation and nucellar embryony (a form of apomixis) have resulted in very low genetic diversity within cultivated citrus types. Wild relatives of plants that are genetically related to many cultivated crops often possess resistance to plant diseases. In stone fruits, hybrid root

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stocks developed using wild relatives of prune are currently used extensively for cultivation of prune, peach, plum, apricot, and almond (Guajardo et al. 2015). Resistance to quick decline caused by the citrus tristeza virus prompted the widespread use of both trifoliolate orange, and its hybrids with citrus as rootstocks. Incorporating useful traits from exotic germplasm can provide solutions to crucial disease problems and alleviate genetic vulnerability (Ford-Lloyd et al. 2011; Holland 2004).

HLB-tolerant cultivars have been identified by various research groups around the world (Koizumi et al. 1993; Shokrollah et al. 2009; Stover and McCollum 2011; Stover et al. 2014). Identification and analysis of HLB resistance in citrus relatives can be valuable both for understanding the mechanisms of resistance as well as for breeding trials. We have conducted an HLB evaluation trial using material from the Citrus Variety Collection (CVC), University of California, Riverside, CA, which has over 1,200 cataloged accessions belonging to the subfamily Aurantioideae. Research and propagation material for many of these accessions are available to researchers throughout the world from the United States Department of Agriculture (USDA), Agricultural Research Service, National Clonal Germplasm Repository for Citrus and Dates, Riverside, CA. Details of each genotype are available from the website (<http://www.citrusvariety.ucr.edu>).

In this study, a long-term field evaluation was conducted to understand the responses of various cataloged genotypes of citrus and citrus relatives to prevalent liberibacter populations associated with HLB in Florida under natural challenge conditions. Any valuable resistance identified in this trial can be traced back to individual trees for further research. The use of seedlings avoided possible influence of rootstocks on disease response; open pollinated seeds increased the probability of introducing variability within each accession, especially in accessions where apomixis does not exist.

The definition of resistance varies in different pathosystems. In general, a resistant plant will have a much lower level of the pathogen compared with a susceptible plant and will not be as symptomatic. Susceptible taxa typically have a higher pathogen titer and will display symptoms. Tolerance is difficult to measure; tolerant plants may have typical disease symptoms but continue to produce fruit. Immunity is defined as a state where the pathogen is never detected in the plant even though it is exposed to the pathogen (Vale et al. 2001).

The main objective of the current study was to identify sources of HLB tolerance or resistance, or both, in citrus cultivars and close relatives belonging to the subfamily Aurantioideae. Since HLB is known to have a long incubation period, the plant hosts often have low pathogen titers and the bacterium is irregularly distributed within the plant host. Hence, we conducted the field trial for a period of six years to enable accurate evaluation of the test accessions. We had three objectives: i) to identify patterns of disease response in cataloged accessions of the plant hosts; ii) to identify sources of disease resistance and tolerance under field conditions in citrus and closely related genera; and iii) to compare HLB incidence over time in specific accessions, with psyllid infestation data that was generated in a different study using the same set of plants. To our knowledge, this is a first report of a large, replicated trial representing genetically diverse accessions that have been systematically screened for the purpose of identifying immune, resistant, and HLB tolerant germplasm.

Materials and Methods

Source of plant materials, experimental design. The majority of the taxa used for the field trial were cataloged accessions obtained from the CVC (<http://www.citrusvariety.ucr.edu>). Seeds were collected from 108 accessions of citrus and citrus relatives belonging to the subfamily Aurantioideae, family Rutaceae. Plants were selected from the core collection to represent about 85% of the genetic diversity present in the entire collection (Barkley 2003). Seeds for *Afraegle paniculata* (Schum.) Engl., *Aegle marmelos* (L.) Corr., and *Casimiroa edulis* La Llave & Lex were obtained from the Fruit and Spice Park, Miami, FL. Seeds of *Zanthoxylum ailanthoides* were obtained from the University of Georgia. Plant maintenance in the

greenhouses was as described in a previous publication (Westbrook et al. 2011). Briefly, the seeds were germinated in cone-tainers in greenhouses located in the U.S. Horticultural Research Laboratory, Fort Pierce, FL. Six- to nine-month-old seedlings were planted in the field in a randomized complete block design in July 2009. Trees were planted with 0.6-m spacing. The field plants were irrigated and fertilized but pesticides were not applied during the study, to encourage psyllid survival, feeding, colonization, and subsequent infection of the plants. Maintenance of the field trees has been described elsewhere (Richardson et al. 2011). The HLB disease pressure was considered to be very high in Pico's farm, Fort Pierce, FL, where the field trial was conducted. The psyllid populations were adequate for conducting a HLB evaluation trial under natural disease spread conditions (Richardson et al. 2011, Westbrook et al. 2011).

Sample collection and DNA extraction. Twigs from three or more branches were collected from individual trees for further processing. If disease symptoms were observed, samples were collected from branches suspected to have the pathogen. We used both petioles (from five to 10 leaves) and bark pieces for the DNA extractions. About 100 mg of chopped tissue was placed in 2-ml screwcap tubes with O-rings (Thermo Fisher Scientific, Fair Lawn, NJ). About 8 to 10 zirconium beads (Glen Mills, Inc., Clifton, NJ) of 2.5 mm diameter were added, the samples were lyophilized overnight, and were then homogenized with extraction buffer (Qiagen, Inc., Valencia, CA) in a bead beater (Biospec Products Inc., Bartlesville, OK). At the beginning of the field trial, samples were processed at the U.S. Horticultural Research laboratory, Fort Pierce, FL, using Plant DNeasy kit (Qiagen, Inc.) according to the manufacturer's instructions. The DNA extractions were shipped to the USDA Riverside laboratory for further analysis. In the last two years of the experiment (2014 and 2015), about 500 mg of petiole, midrib, and shoot samples were placed in 2-ml screwcap tubes, filled with 95% alcohol, and were shipped to Riverside under a valid USDA APHIS (Animal and Plant Health Inspection Service) moving permit. Alcohol-preserved samples were carefully removed from the tubes, air-dried briefly for about 3 min, and, using a razor blade, about 100 mg of sample representing petiole, midrib, and bark tissue was chopped and added to 96 individual wells of a deep-well plate (USA Scientific Inc., Ocala, FL) along with 2.5 mm of zirconium beads (about 8 to 10) and covered with cluster caps (USA Scientific Inc., Ocala, FL). In each 96-well plate, four wells (F12 to H12) were designated for positive and negative controls in polymerase chain reaction (PCR). In addition, three to five randomly selected wells without plant samples served as negative extraction controls. Care was taken to avoid cross contaminations by changing gloves between samples. Plant material was removed using forceps and all tools were bleached for a minimum of 3 min between samples, followed by a sterile water rinse. The lyophilized samples were powdered using a bead beater twice, 3 min each, and centrifuged for 5 min at $3,750 \times g$. Cluster caps from individual columns were removed carefully and 500 μ l of RLT buffer (Qiagen, Inc.) was added. The caps were replaced and the samples were ground again, using the bead beater as described above. Further processing was done robotically, according to the manufacturer's instructions, in a KingFisher flex magnetic particle processor (Thermo Scientific), and the extracted DNA was eluted in a final volume of 100 μ l of AE buffer (Qiagen, Inc.).

Quantitative (q)PCR methods. We used a TaqMan-based multiplex real-time qPCR method for detection of the 16S rDNA gene from '*Ca. L. asiaticus*' and the mitochondrial gene cytochrome oxidase (COX) from the plant hosts (Li et al. 2006; Manjunath et al. 2008; Razi et al. 2014). Cycle threshold (Ct) values for the COX gene were useful to verify quality of DNA extractions. Inclusion of positive plasmid control samples and negative extraction controls was useful in maintaining rigorous quality control during extractions and qPCR assays. A Stratagene Mx3005P qPCR machine (Agilent Technologies, Santa Clara, CA) and an Applied Biosystems ViiA 7 real-time PCR system (Thermo Scientific) were utilized for the qPCR assays.

To confirm tolerance/resistance recorded in several individual trees, five to 10 additional samples were collected from multiple branches, DNA extractions and qPCR were conducted separately for the different samples. Only one value for each time point (the lowest Ct recorded by qPCR analysis) is indicated in the data tables (Supplementary Table S1).

Plant response categories. Instead of determining if the plants are susceptible or resistant, we assigned plants to eight different

categories depending on the qPCR results, intensity of HLB symptoms, and plant growth patterns in the field, as described below.

Results

HLB disease categories. Most studies describing HLB disease status of citrus and related accessions categorize the host plants as susceptible, tolerant, or resistant. In the current study, which includes heterologous taxa belonging to 21 genera, we identified eight types

Table 1. Taxa evaluated in the field trial and the huanglongbing disease rating based on disease symptoms and pathogen titer, as estimated by real time polymerase chain reaction assays

No.	Botanical name (CRC number)	Common/cultivar name	Taxonomical group	Category
1	<i>Clausena excavata</i> Burm. f. (3166)	Pink wampee	Aur ^a /Clauseneae/Clauseninae	1
2	<i>Glycosmis pentaphylla</i> (Retz.) DC. (3285)	Orange berry/Gin berry	Aur/Clauseneae/Clauseninae	1
3	<i>Bergera koenigii</i> L. (3165)	Curry leaf	Aur/Clauseneae/Clauseninae	2
4	<i>Casimiroa edulis</i> La Llave & Lex	White sapote	Toddalioideae	2
5	<i>Eremocitrus glauca</i> hybrid (Lindl.) Swingle (4105)	Australian desert lime hybrid	Aur/Citreae/Citrinae	2
6	<i>Murraya paniculata</i> (L.) Jack. (1637)	'Orange jasmine'	Aur/Clauseneae/Clauseninae	2
7	<i>Naringi crenulata</i> (Roxb.) Nicolson (2879)	Hesperethusa	Aur/Citreae/Citrinae	2 ^b
8	<i>Zanthoxylum ailanthoides</i> Siebold & Zucc.	Japanese prickly ash	Toddalioideae	2
9	<i>Citrus halimii</i> B.C. Stone (3780)	'Mountain' citron	Aur/Citreae/Citrinae	3
10	<i>Microcitrus australasica</i> (F. Muell.) Swing. (1484)	Australian finger lime var. 'Sanguinea'	Aur/Citreae/Citrinae	3
11	<i>Microcitrus</i> hybrid (<i>M. australis</i> × <i>M. australasica</i>) (1485)	'Sydney hybrid'	Aur/Citreae/Citrinae	3
12	<i>Microcitrus inodora</i> (F.M.Bail) Swing. (3785)	Large-leaf Australian wild lime	Aur/Citreae/Citrinae	3*
13	<i>XMicrocitronella</i> sp. (<i>Microcitrus australasica</i> × <i>Calamondin</i>) (1466)	'Faustrimedini'	Aur/Citreae/Citrinae	3
14	<i>Poncirus trifoliata</i> L. (3549)	'Simmon's trifoliata' hybrid	Aur/Citreae/Citrinae	3
15	<i>Aegle marmelos</i> (L.) Corr.	Indian bael fruit	Aur/Citreae/Balsamocitrinae	4
16	<i>Aeglopsis chevalieri</i> Swingle (2878)	Chevalier's Aeglopsis	Aur/Citreae/Balsamocitrinae	4
17	<i>Balsamocitrus dawsonii</i> Stapf. (3514)	Uganda powder flask	Aur/Citreae/Balsamocitrinae	4
18	<i>Citrus latipes</i> (Swing.) Tan. (3052)	'Khasi' papeda	Aur/Citreae/Citrinae	4
19	<i>Microcitrus australis</i> (A. Cunn. ex Mudie) Swingle (3673)	Australian round lime	Aur/Citreae/Citrinae	4
20	<i>Murraya paniculata</i> (L.) Jack. (3171)	'Hawaiian mock orange' var. 'Ovatifoliolata'	Aur/Clauseneae/Clauseninae	4
21	<i>Poncirus trifoliata</i> L. (4007)	'Little-leaf' trifoliata	Aur/Citreae/Citrinae	5
22	<i>XCitroncirus</i> sp. (<i>Cleopatra mandarin</i> × <i>P. trifoliata</i>) (3957)	'X639' trifoliata hybrid	Aur/Citreae/Citrinae	5
23	<i>Citrus amblycarpa</i> Och. (2485)	'Nasranan' mandarin	Aur/Citreae/Citrinae	6
24	<i>Citrus aurantifolia</i> (Christm.) Swing. (2450)	'India' lime	Aur/Citreae/Citrinae	6
25	<i>Citrus aurantium</i> L. (2717)	'Olivelands' sour orange	Aur/Citreae/Citrinae	6
26	<i>Citrus aurantium</i> L. (628)	'Standard' sour orange	Aur/Citreae/Citrinae	6
27	<i>Citrus dawaensis</i> (Wester) Tan. (2427)	'Davao' lemon	Aur/Citreae/Citrinae	6
28	<i>Citrus excelsa</i> Wester (2317)	'Limon Real' papeda	Aur/Citreae/Citrinae	6
29	<i>Citrus hassaku</i> hort. ex Tan. (3907)	'Hassaku' pummelo hybrid	Aur/Citreae/Citrinae	6
30	<i>Citrus hassaku</i> hort ex Tan (3942)	'Hassaku' pummelo hybrid	Aur/Citreae/Citrinae	6
31	<i>Citrus</i> hybrid (53-1-16 'Clem' × 'Hamlin') × Chinotto F1 (3715)	Ex-India sour orange hybrid	Aur/Citreae/Citrinae	6
32	<i>Citrus jambhiri</i> Lush. (400)	'Florida' rough lemon	Aur/Citreae/Citrinae	6
33	<i>Citrus leiocarpa</i> hort. Ex Tan. (3147)	'Koji' mandarin	Aur/Citreae/Citrinae	6
34	<i>Citrus limettoides</i> Tan. (1482)	'Palestine' sweet lime	Aur/Citreae/Citrinae	6
35	<i>Citrus limon</i> (L.) Burm. f. (3005)	'Frost Nucellar Eureka' lemon	Aur/Citreae/Citrinae	6
36	<i>Citrus limon</i> (L.) Burm. f. (3593)	'Interdonato' lemon	Aur/Citreae/Citrinae	6
37	<i>Citrus limon</i> (L.) Burm. f. (3885)	Iranian lemon	Aur/Citreae/Citrinae	6
38	<i>Citrus limonia</i> (L.) Osbeck (3919)	'Lamas' lemon	Aur/Citreae/Citrinae	6
39	<i>Citrus limonia</i> Osbeck. (712)	'Santa Barbara' red lime	Aur/Citreae/Citrinae	6
40	<i>Citrus longispina</i> Wester (2320)	'Talamisan'; 'Winged' lime	Aur/Citreae/Citrinae	6
41	<i>Citrus macrophylla</i> Wester (3842)	'Alemow' hybrid	Aur/Citreae/Citrinae	6
42	<i>Citrus maxima</i> (Burm.) Merr. (2242)	'Kao Pan' pummelo	Aur/Citreae/Citrinae	6
43	<i>Citrus maxima</i> (Burm.) Merr. (2248)	'Kao Panne' pummelo	Aur/Citreae/Citrinae	6
44	<i>Citrus maxima</i> (Burm.) Merr. (3805)	'Reinking' pummelo	Aur/Citreae/Citrinae	6
45	<i>Citrus medica</i> L. (3523)	'Diamante' citron	Aur/Citreae/Citrinae	6
46	<i>Citrus medica</i> L. (3546)	'South Coast Field Station' citron	Aur/Citreae/Citrinae	6

(continued on next page)

^a Aur = Aurantioideae.

^b Asterisks (*) indicate insufficient data.

of responses to the HLB challenge. Real time Ct cutoff values were not set to determine the disease categories. Instead, the complete absence of the pathogen (immunity), absence of pathogen colonization in spite of transient replication (resistance), and successful colonization of the pathogen leading to disease symptom expression (susceptibility) were considered in determining disease categories. Tolerant categories included those accessions in which plants were able to recover from disease and in which the onset of disease was delayed. Another category of tolerant accessions exhibited

variability among test plants, with some being susceptible and others resistant. Most accessions in the last category were seedlings of known hybrids.

The field trial was first started with 108 accessions and about 886 seedlings. At the end of six years, 419 plants survived. We were able to grow 98 accessions with replications and retrieved meaningful data from about 90 accessions (data obtained from more than one plant in year two). Many noncitrus accessions did not perform well in the field trial, since growth conditions were not optimal and

Table 1. (continued from preceding page)

No.	Botanical name (CRC number)	Common/cultivar name	Taxonomical group	Category
47	<i>Citrus medica</i> L. (661)	'Indian' citron hybrid	Aur/Citreae/Citrinae	6
48	<i>Citrus neo-aurantium</i> (C.obovoidea + C. unshiu graft chimera) (3816)	'Kinkoji Unshiu' graft chimera	Aur/Citreae/Citrinae	6
49	<i>Citrus neo-aurantium</i> Tanaka (3611)	'Konejime' sour orange hybrid	Aur/Citreae/Citrinae	6
50	<i>Citrus reticulata</i> Blanco (3558)	'Fremont' mandarin	Aur/Citreae/Citrinae	6
51	<i>Citrus</i> sp. (3172)	Unnamed lemon hybrid	Aur/Citreae/Citrinae	6
52	<i>Citrus taiwanica</i> Tan. and Shimada (2588)	'Nasho Daidai' sour orange	Aur/Citreae/Citrinae	6
53	<i>Citrus volkameriana</i> Osbeck (3050)	'Volkamer' lemon hybrid	Aur/Citreae/Citrinae	6
54	<i>Citrus webberi</i> Wester (1455)	'Kalpi' papeda	Aur/Citreae/Citrinae	6
55	<i>Severinia buxifolia</i> (Poiret) Tan. (1497)	Chinese box orange	Aur/Citreae/Citrinae	6
56	<i>Severinia buxifolia</i> (Poiret) Tan. (4107)	Chinese box orange	Aur/Citreae/Citrinae	6
57	<i>Severinia buxifolia</i> (Poiret) Ten. (1491)	Chinese box orange	Aur/Citreae/Citrinae	6
58	<i>XCitroncirus</i> sp. (301)	'Rusk' citrange trifoliolate hybrid	Aur/Citreae/Citrinae	6
59	<i>XCitroncirus</i> sp. (3552)	'S-281' citrangelo	Aur/Citreae/Citrinae	6
60	<i>XCitroncirus</i> sp. (C. paradisi 'Duncan' × P. trifoliata) (3771)	'Swingle' citrumelo	Aur/Citreae/Citrinae	6
61	<i>Citrus aurantifolia</i> (Christm.) Swing. (3822)	'Mexican' lime	Aur/Citreae/Citrinae	7
62	<i>Citrus aurantium</i> L. (3929)	'Goutoucheng' sour orange	Aur/Citreae/Citrinae	7
63	<i>Citrus aurantium</i> L. (3930)	'Zhuluan' sour orange hybrid	Aur/Citreae/Citrinae	7
64	<i>Citrus benikoji</i> hort. Ex Tan. (3149)	Unnamed tangor	Aur/Citreae/Citrinae	7
65	<i>Citrus intermedia</i> hort. Ex Tan. (3474)	'Yama-mikan' sour orange	Aur/Citreae/Citrinae	7
66	<i>Citrus limon</i> (L.) Burm. f. (3176)	'Frost Nucellar Lisbon' lemon	Aur/Citreae/Citrinae	7
67	<i>Citrus limon</i> (L.) Burm. f. (3892)	'Mesero' lemon	Aur/Citreae/Citrinae	7
68	<i>Citrus lycopersiciformis</i> hort. ex Tan. (3564)	Kokni orange	Aur/Citreae/Citrinae	7
69	<i>Citrus maderasapata</i> (3225)	'Kitchli' sour orange hybrid	Aur/Citreae/Citrinae	7 ^b
70	<i>Citrus maxima</i> (Burm.) Merr. (3945)	'Mato Buntan' pummelo	Aur/Citreae/Citrinae	7
71	<i>Citrus maxima</i> (Burm.) Merr. (3959)	'Egami Buntan' pummelo	Aur/Citreae/Citrinae	7
72	<i>Citrus maxima</i> (Burm.) Merr. (4026)	'Pomelit' pummelo hybrid	Aur/Citreae/Citrinae	7
73	<i>Citrus paradisi</i> Macf. (3781)	'Tahitian' pummelo × 'Star Ruby' grapefruit	Aur/Citreae/Citrinae	7
74	<i>Citrus reticulata</i> Blanco (3018)	'Dweet' tangor	Aur/Citreae/Citrinae	7
75	<i>Citrus reticulata</i> Blanco (2590)	'Tien Chieh' mandarin	Aur/Citreae/Citrinae	7
76	<i>Citrus reticulata</i> Blanco (3022)	'Frua' mandarin	Aur/Citreae/Citrinae	7
77	<i>Citrus reticulata</i> Blanco (3260)	'Soh Niamtra' mandarin	Aur/Citreae/Citrinae	7
78	<i>Citrus reticulata</i> Blanco (3326)	'Scarlett Emperor' mandarin	Aur/Citreae/Citrinae	7
79	<i>Citrus reticulata</i> Blanco (3363)	'Belady' mandarin	Aur/Citreae/Citrinae	7
80	<i>Citrus reticulata</i> Blanco (3752)	'Som Keowan' mandarin	Aur/Citreae/Citrinae	7
81	<i>Citrus reticulata</i> Blanco (3958)	'Koster' mandarin	Aur/Citreae/Citrinae	7
82	<i>Citrus reticulata</i> Blanco (4003)	'Sun Chu Sha' mandarin	Aur/Citreae/Citrinae	7
83	<i>Citrus reticulata</i> hybrid (Clementine × Orlando) (3850)	'Robinson' mandarin	Aur/Citreae/Citrinae	7
84	<i>Citrus sinensis</i> (L.) Osbeck (3858)	'Pineapple' sweet orange	Aur/Citreae/Citrinae	7
85	<i>Citrus sunki</i> hort. ex Tan. (3143)	'Sunki' mandarin	Aur/Citreae/Citrinae	7
86	<i>Citrus aurantium</i> L. (3289)	Sour orange var. salicifolia	Aur/Citreae/Citrinae	8
87	<i>Citrus nobilis</i> Lour. (3845)	'King' mandarin	Aur/Citreae/Citrinae	8
88	<i>Citrus reticulata</i> Blanco (300)	'Parson's Special' mandarin	Aur/Citreae/Citrinae	8
89	<i>Citrus reticulata</i> Blanco (3812)	Unnamed mandarin	Aur/Citreae/Citrinae	8
90	<i>Citrus reticulata</i> hybrid (Clementine × Orlando) (3851)	'Lee' mandarin	Aur/Citreae/Citrinae	8
91	<i>Afraegle paniculata</i> (Schum.) Engl.	Nigerian Powder flask		*
92	<i>Atalantia ceylanica</i> (Am.) Oliv. (3725)	Atalantia	Aur/Citreae/Citrinae	*
93	<i>Citropsis dawean</i> Swing. & M. Kell (3294)	Citropsis	Aur/Citreae/Citrinae	*
94	<i>Citrus reticulata</i> Blanco (3991)	'USDA 88-2' mandarin	Aur/Citreae/Citrinae	*
95	<i>Clausena harmandiana</i> Pierre ex Guillaumin (4034)	Clausena	Aur/Clauseneae/Clauseninae	*
96	<i>Fortunella hindsii</i> Champ. ex Benth. (3789)	Unnamed kumquat	Aur/Citreae/Citrinae	*
97	<i>Severinia buxifolia</i> (Poiret) Tan. (1495)	Chinese box orange	Aur/Citreae/Citrinae	*
98	<i>Severinia buxifolia</i> (Poiret) Tan. (3724)	Chinese box orange	Aur/Citreae/Citrinae	*

plant-damaging freezes occurred in the first two years. For certain taxa, data were obtained from only a few plants, making the disease category determinations challenging. These are indicated in the tables as insufficient data in the disease category column. Table 1 has a list of accessions included in the field trial, their scientific and common names, taxonomical groups and assigned HLB disease categories. The complete qPCR data indicating Ct values of all test plants over a period of six years is shown in data tables.

Response of accessions to HLB exposure by disease category.
Category 1. Two accessions, *Clausena excavata* (pink wampee) and *Glycosmis pentaphylla* (orange berry) were considered immune.

Despite repeated testing of multiple samples for a period of six years, we were never able to detect '*Ca. L. asiaticus*' in either accession.

Category 2. Six accessions were considered resistant: *Berbera koenigii* (curry leaf), *Casimiroa edulis* (white sapote), *Eremocitrus glauca* (Australian desert lime), *Murraya paniculata* ('Orange jasmine', which was recently classified as *Murraya exotica* [A. Beattie, personal communication]), *Naringi crenulata* (Hesperethusa), and *Zanthoxylum ailanthoides* (Japanese prickly ash). Even though taxa assigned to this group were able to support replication of the pathogen transiently, the liberibacter bacterium could not establish in these hosts, although a low level of replication of the bacterium was

Table 2. Huanglongbing disease category based on pathogen titers, as estimated by annual real time polymerase chain reaction results (cycle threshold [Ct] values)^a

No.	Common name of taxa (CRC number) ^a	Category ^b	Ct range for ' <i>Candidatus L. asiaticus</i> ' (number of plants) ^c					Asian citrus psyllid colonization ^d
			Year 1	Year 2	Year 3	Year 4	Year 6	
1	Pink wampee (3166)	1	40 (8)	40 (7)	40 (6)	40 (6)	40 (6)	0.56/0.94
2	Orange berry (3285)	1	40 (8)	40 (8)	40 (8)	40 (8)	40 (8)	0.16/0.03
3	Curry leaf (3165) (P)	2	40 (8)	40 (7)	36–40 (7)	40 (7)	40 (7)	1.35/1.81
4	White sapote	2	31–40 (8)	37–40 (8)	40 (8)	40 (8)	39–40 (7)	0.00/0.00
5	Australian desert lime hybrid (4105) (M)	2	36–40 (8)	35–40 (7)	40 (7)	40 (7)	35–40 (5)	0.20/0.90
6	'Orange jasmine' (1637)	2	38–40 (8)	35–40 (5)	40 (5)	40 (5)	40 (5)	1.07/1.48
7	Hesperethusa (2879)	2*	37–40 (7)	40 (2)	36–40 (2)	Dead	Dead	NA
8	Japanese prickly ash	2	40 (7)	40 (7)	40 (5)	40 (3)	39–40 (2)	0.29/0.00
9	'Mountain' citron (3780)	3	38–40 (8)	38–40 (4)	29–40 (4)	28–40 (2)	40 (1)	0.27/0.64
10	Australian finger lime var. 'Sanguinea' (1484) (PM)	3	30–40 (6)	30–40 (6)	36–40 (5)	31–40 (5)	30–40 (5)	0.24/0.48
11	'Sydney hybrid' (1485) (PM)	3	27–40 (8)	30–40 (8)	28–40 (8)	33–40 (7)	29–37 (6)	0.26/1.68
12	Large-leaf Australian wild lime (3785) (PM)	3*	34–40 (6)	37 (2)	30 (1)	Dead		0.14/0.86
13	'Faustmedin' (1466)	3	37–40 (8)	25–40 (6)	24–40 (4)	40 (1)	40 (1)	0.27/0.65
14	'Simmon's trifoliolate' (3549) (PP)	3	40 (8)	32–40 (8)	35–40 (7)	28–40 (7)	40 (6)	0.07/0.04
15	Indian bael fruit (P)	4	27–40 (5)	27–32 (4)	31–34 (4)	33–37 (4)	36–40 (4)	0.27/0.50
16	Chevalier's Aeglopsis (2878)	4	40 (3)	28–40 (3)	39–40 (3)	40 (1)	40 (1)	NA
17	Uganda powder flask (3514)	4	28–40 (8)	30–40 (7)	31–40 (6)	40 (6)	40 (6)	0.35/0.62
18	'Khasi' papada (3052)	4	30–40 (8)	24–40 (7)	23–40 (6)	31–40 (6)	40 (6)	0.42/1.04
19	Australian round lime (3673)	4	26–40 (8)	28–40 (8)	29–40 (7)	31–40 (6)	30–40 (6)	0.38/0.92
20	'Hawaiian mock orange' (3171)	4	28–40 (8)	35–40 (8)	35–40 (8)	40 (8)	40 (7)	0.70/1.20
21	'Little-leaf' trifoliolate (4007) (P)	5	32–40 (8)	29–40 (8)	26–40 (8)	28–40 (8)	28–40 (8)	0.06/0.03
22	'X639' trifoliolate hybrid (3957) (P)	5	32–40 (8)	30–40 (8)	25–40 (8)	27–36 (8)	27–34 (8)	0.39/0.77
23	'Nasnaran' mandarin (2485) (P)	6	22–40 (8)	22–27 (8)	23–27 (7)	27–29 (7)	26–31 (7)	0.62/1.28
24	'India' lime (2450) (P)	6	22–40 (8)	23–30 (7)	22–28 (7)	26–31 (7)	26–33 (7)	0.83/1.79
25	'Oliveland's' sour orange (2717) (PP)	6	38–40 (8)	23–29 (8)	24–29 (8)	25–29 (8)	28–33 (8)	0.76/1.72
26	'Standard' sour orange (628) (P)	6	26–40 (8)	22–28 (8)	22–28 (8)	24–30 (8)	28–35 (8)	0.29/1.14
27	'Davao' lemon (2427)	6	25–40 (8)	25–32 (7)	23–31 (7)	27–32 (7)	23–33 (7)	0.78/1.70
28	'Limon Real' papada (2317)	6	21–40 (8)	26–34 (8)	26–31 (8)	27–32 (8)	29–35 (8)	0.62/1.58
29	'Hassaku pummelo' hybrid (3907)	6	20–40 (8)	21–33 (8)	24–40 (8)	23–36 (7)	21–40 (6)	0.54/1.77
30	'Hassaku pummelo' hybrid (3942)	6	20–40 (8)	21–31 (8)	23–31 (8)	24–35 (7)	25–35 (7)	0.73/1.83
31	Ex-India sour orange hybrid (3715)	6	20–40 (8)	23–26 (6)	25–40 (4)	26–29 (3)	26–31 (3)	0.76/1.36
32	'Florida' rough lemon (400)(P)	6	26–40 (8)	23–37 (8)	24–28 (7)	25–29 (6)	27–31 (6)	0.53/1.37
33	'Koji' mandarin (3147)	6	21–40 (8)	25–40 (7)	21–27 (6)	25–31 (6)	24–29 (6)	0.25/0.71
34	'Palestine' sweet lime (1482) (P)	6	23–40 (8)	26–40 (8)	24–28 (7)	25–29 (5)	28–30 (5)	0.75/1.88
35	'Frost Nucellar Eureka' lemon (3005) (P)	6	22–40 (7)	28–40 (7)	26–31 (7)	27–29 (4)	28–35 (4)	0.77/1.38
36	'Interdonato' lemon (3593)	6	22–40 (8)	24–30 (6)	26–33 (6)	28–31 (6)	27–30 (6)	0.59/1.66
37	Iranian lemon (3885) (PP)	6	22–28 (8)	22–32 (7)	23–31 (7)	26–31 (6)	27–34 (6)	0.85/1.81
38	'Lamas' lemon (3919) (PP)	6	22–40 (8)	28–40 (5)	25–33 (5)	29–30 (4)	28–34 (4)	0.61/1.89
39	'Santa Barbara' red lime (712) (P)	6	21–34 (8)	25–35 (8)	26–30 (8)	28–31 (8)	29–33 (8)	0.81/1.94
40	'Winged' lime (2320)	6	31–40 (8)	21–40 (7)	22–32 (6)	27–31 (4)	29–33 (4)	0.37/1.04
41	'Alemow' hybrid (3842) (P)	6	21–40 (8)	27–40 (8)	28–40 (8)	28–39 (8)	31–34 (8)	0.83/1.72
42	'Kao Pan' pummelo (2242) (M)	6	23–40 (8)	19–24 (8)	21–34 (7)	28–31 (7)	22–40 (7)	0.56/1.67
43	'Kao Panne' pummelo (2248) (M)	6	31–40 (8)	22–40 (7)	22–30 (7)	24–29 (7)	24–35 (7)	0.61/1.75
44	'Reinking' pummelo (3805) (M)	6	22–40 (8)	23–31 (6)	20–40 (6)	22–30 (4)	24–32 (4)	0.57/1.36
45	'Diamante' citron (3523) (M)	6	20–40 (8)	26–32 (8)	23–33 (8)	27–34 (8)	28–34 (8)	0.97/1.81
46	'South Coast Field Station' citron (3546) (M)	6	21–30 (8)	25–35 (8)	20–33 (7)	26–32 (5)	31–32 (5)	0.86/2.07

(continued on next page)

^a (M) = Monoembryonic; (PM) = presumed to be monoembryonic; (P) = polyembryonic; (PP) = presumed to be polyembryonic.

^b Asterisks (*) indicate insufficient data for disease rating.

^c Range of Ct values for five years indicated. Numbers in parentheses after the Ct values indicate number of living plants at each time point from which data were recorded. Psyllid colonization data are from Westbrook et al. 2011.

^d Mean rating of adults/nymphs was determined on a scale of 0 to 3 (for adults: 0 = no psyllids, 1 = 1 to 5, 2 = 6 to 15, 3 = >15; for nymphs: 0 = no nymphs, 1 = 1 to 10, 2 = 11 to 30, 3 = >30). NA = not available.

detected periodically. Death of many plants in the resistant category was attributed to cold weather during the early part of the field trial (2009 and 2010 winters) and not because of HLB disease issues (Inch et al. 2014).

Bergera koenigii (curry leaf) is a commonly cultivated plant and is well known to be a preferred host for the psyllid (Damsteegt et al. 2010; Westbrook et al. 2011) (Table 2). In the current field trial, we detected the HLB pathogen in curry leaf only once in six years. White sapote, cultivated for its edible fruit, was mostly pathogen-free. Australian desert lime was resistant with a transient, low level of '*Ca. L. asiaticus*' replication (five samples had Ct values ranging

from 35 to 39). In the current field trial, 'Orange jasmine' was considered to be a resistant plant, even though '*Ca. L. asiaticus*' Ct values of 35 to 38 were recorded in six of 28 data points. The overall appearance of 'Orange jasmine' was that of a healthy tree with generally lush green foliage. The pathogen could be detected from symptomatic leaves showing slight blotchy mottle and yellowing of the foliage. However, the pathogen never established and the trees were regarded as healthy in the experimental field with high incidence of HLB. Most trees of the tropical species *Hesperethusa* died in the first two years, probably due to environmental factors. Ct values of 37 to 38 were recorded from four trees in years 1 to 3, indicating brief

Table 2. (continued from preceding page)

No.	Common name of taxa (CRC number) ^a	Category ^b	Ct range for ' <i>Candidatus L. asiaticus</i> ' (number of plants) ^c					Asian citrus psyllid colonization ^d
			Year 1	Year 2	Year 3	Year 4	Year 6	
47	'Indian' citron hybrid (661)	6	23–40 (8)	26–37 (8)	29–36 (8)	24–35 (8)	30–36 (7)	0.73/1.54
48	'Kinkoji Unshiu' (3816) (PP)	6	26–40 (8)	22–27 (8)	22–25 (8)	26–28 (7)	25–33 (7)	0.68/1.75
49	'Konejime' sour orange hybrid (3611) (PP)	6	20–40 (8)	22–28 (8)	23–29 (8)	25–28 (6)	27–30 (6)	0.42/1.54
50	'Fremont' mandarin (3558)	6	21–40 (8)	21–26 (8)	20–24 (6)	26–27 (3)	26–28 (3)	0.76/1.40
51	Unnamed lemon hybrid (3172)	6	25–40 (8)	26–35 (7)	26–31 (7)	26–32 (7)	23–32 (7)	0.72/1.28
52	'Nasho Daidai' sour orange (2588) (P)	6	27–40 (8)	21–28 (7)	23–27 (7)	26–34 (7)	26–40 (7)	0.48/2.07
53	'Volkamer' lemon hybrid (3050) (P)	6	19–32 (7)	22–28 (7)	25–29 (7)	24–30 (6)	28–37 (6)	0.73/1.69
54	'Kalpi' papeda (1455)	6	27–40 (8)	27–40 (7)	28–30 (4)	29–32 (4)	31–34 (4)	0.45/1.66
55	Chinese Box orange (1497) (M)	6	28–40 (8)	26–40 (8)	23–33 (8)	26–31 (8)	26–40 (8)	0.52/0.48
56	Chinese Box orange (4107) (M)	6	36–40 (5)	39–40 (4)	20–30 (4)	26–31 (4)	28 (4)	NA
57	Chinese Box orange (1491) (M)	6	39–40 (4)	31–40 (4)	27–36 (4)	29–30 (3)	30–40 (3)	NA
58	'Rusk' citrange (301) (P)	6	32–40 (8)	21–40 (8)	22–28 (8)	25–27 (8)	27–29 (8)	0.43/1.27
59	'S-281' citrangelo (3552)	6	28–40 (8)	25–40 (8)	25–36 (7)	26–32 (3)	29–33 (3)	0.66/1.55
60	'Swingle' citrumelo (3771) (P)	6	28–40 (8)	22–40 (8)	23–26 (8)	24–29 (8)	29–33 (8)	0.56/2.09
61	'Mexican' lime (3822) (P)	7	22–40 (7)	28–30 (3)	26–28 (3)	24–29 (3)	24–31 (3)	0.57/1.93
62	'Goutoucheng' sour orange (3929) (PP)	7	23–40 (8)	23–30 (8)	23–28 (8)	22–30 (7)	24–32 (7)	0.70/1.30
63	'Zhuluan' sour orange hybrid (3930) (PP)	7	21–40 (7)	22–40 (7)	22–29 (5)	24–30 (5)	26–33 (5)	0.48/1.48
64	Unnamed tangor (3149)	7	20–40 (8)	22–37 (4)	24–27 (4)	25–28 (3)	25–29 (3)	0.54/1.04
65	'Yama-mikan' sour orange (3474)	7	32–40 (7)	21–28 (7)	22–30 (6)	25–30 (5)	27–31 (5)	0.52/1.15
66	'Frost Nucellar Lisbon' lemon (3176) (P)	7	23–40 (8)	27–40 (7)	27–30 (6)	29 (3)	29–32 (3)	0.81/1.73
67	'Mesero' lemon (3892)	7	24–40 (8)	28–40 (8)	28–40 (6)	29–31 (2)	33 (2)	0.63/1.41
68	Kokni orange (3564) (P)	7	23–40 (8)	25–35 (7)	24–29 (4)	30 (1)	34 (1)	0.46/1.00
69	'Kitchli' sour orange (3225)	7*	36–40 (7)	36 (1)	Dead			NA
70	'Mato Buntan' pummelo (3945) (M)	7	21–40 (7)	22–36 (7)	23–40 (5)	25–27 (2)	26–31 (2)	0.90/1.38
71	'Egami Buntan' pummelo (3959) (M)	7	22–40 (8)	21–40 (6)	22–27 (5)	26–31 (5)	23–40 (5)	0.50/1.60
72	'Pomelit' pummelo hybrid (4026)	7	21–40 (8)	22–27 (8)	22–29 (7)	25–30 (6)	27–34 (6)	0.55/1.72
73	'Tahitian' pummelo × 'Star Ruby' grapefruit (3781)	7	20–40 (8)	22–30 (7)	24–40 (6)	27–29 (2)	28–30 (2)	0.63/1.89
74	'Dweet' tangor (3018) (P)	7	28–40 (8)	23–40 (8)	22–28 (5)	26–28 (4)	25–27 (3)	0.47/1.27
75	'Tien Chieh' mandarin (2590)	7	40 (8)	20–24 (8)	19–23 (6)	24–27 (2)	23–24 (2)	1.11/1.63
76	'Frua' mandarin (3022) (P)	7	21–40 (5)	21–28 (2)	21 (1)	Dead		0.73/1.27
77	'Soh Niamtra' mandarin (3260)	7	19–40 (8)	20–24 (7)	20–24 (4)	23–24 (3)	22–26 (3)	0.87/1.65
78	'Scarlett Emperor' mandarin (3326)	7	19–40 (8)	23–33 (5)	23–27 (4)	25–28 (4)	27–30 (4)	0.56/0.96
79	'Belady' mandarin (3363)	7	34–40 (8)	21–39 (7)	19–28 (6)	20–26 (4)	24–40 (3)	0.52/1.23
80	'Som Keowan' mandarin (3752)	7	21–40 (8)	21–26 (8)	22–27 (5)	25–27 (4)	26–34 (4)	0.75/1.57
81	'Koster' mandarin (3958)	7	19–40 (8)	24–40 (6)	20–40 (5)	30 (1)	37 (1)	0.69/1.72
82	'Sun Chu Sha' mandarin (4003) (P)	7	33–40 (8)	20–25 (8)	21–26 (7)	23–25 (4)	22–27 (4)	0.55/1.62
83	'Robinson' mandarin (3850) (M)	7	25–40 (8)	20–40 (8)	20–25 (5)	24–25 (4)	24–27 (4)	0.55/0.90
84	'Pineapple' sweet orange (3858) (P)	7	24–40 (8)	22–35 (7)	23–26 (5)	23–27 (4)	30–38 (2)	0.39/1.21
85	'Sunki' mandarin (3143) (P)	7	26–40 (8)	23–37 (7)	24–27 (3)	26 (2)	27–29 (2)	0.80/1.37
86	Sour orange var. <i>salicifolia</i> (3289) (PP)	8	34–40 (3)	24–28 (2)	23–25 (2)	28 (1)	32 (1)	0.43/1.00
87	'King' mandarin (3845) (P)	8	39–40 (8)	19–40 (4)	21 (1)	Dead		0.52/1.11
88	'Parson's Special' mandarin (300)	8	22–40 (8)	21–40 (5)	21–23 (2)	Dead		0.54/0.93
89	Unnamed mandarin (3812)	8	23–40 (8)	22–26 (5)	22–30 (3)	26 (1)	26 (1)	0.52/1.00
90	'Lee' mandarin (3851) (M)	8	21–40 (7)	19–40 (5)	22–23 (2)	26 (1)	25 (1)	0.43/1.04
91	Nigerian Powder flask	*	38–40 (8)	Dead				0.55/1.26
92	Atalantia (3725)	*	Dead					NA
93	Citropsis (3294)	*	40 (3)	Dead				NA
94	USDA 88-2 (3991)	*	Dead					NA
95	<i>Clausena harmandiana</i> (4034)	*	40 (7)	35 (1)	Dead			0.19/0.15
96	Unnamed kumquat (3789) (P)	*	Dead					NA
97	Chinese box orange (1495) (M)	*	38–40 (2)	Dead				NA
98	Chinese box orange (3724) (M)	*	Dead					NA

periods of pathogen replication. In Japanese prickly ash, belonging to the subfamily Toddalioidae, one sample had a Ct value of 39 (out of a total of 24 samples).

Category 3. Accessions in this category consisted of HLB tolerant taxa that showed different disease responses within the eight test plants belonging to one accession, probably due to seedling variations (Table 2). *Citrus halimii* ('Mountain' citron), *Microcitrus australasica* (Australian finger lime), *Microcitrus* hybrid ('Sydney hybrid'), *Microcitrus inodora* (Large-leaf Australian wild lime), *Microcitronella* sp. ('Faustrimedin'), and *Poncirus trifoliata* ('Simmon's trifoliata') were grouped in this category. Most accessions in this group are presumed to be monoembryonic. It is conceivable that the zygotic seedlings may have genetic differences that may result in nonuniform disease response phenotypes in the eight plants of one accession tested. In addition, the progeny of known hybrids ('Sydney hybrid' and 'Faustrimedin') showed segregation of characters. 'Simmon's trifoliata' is assumed to be polyembryonic (M. L. Roose, unpublished information) and also is reported to be a nonpreferred host for the psyllid (Westbrook et al. 2011). Under field conditions, several other factors, such as freeze damage, canker, and other biotic and abiotic causes, also played a role in the death of plants. We determined the probable cause of death of individual plants on a case-by-case basis, mainly depending on apparent disease status before a plant died.

The 'Mountain' citron did not perform well in the Fort Pierce field, since four of the eight plants died after year 1, probably due to environmental factors. Two of the surviving plants died during years 4 and 5, presumably because of HLB, since Ct values of 27 to 28 were recorded from the samples collected and symptoms of HLB were observed. However, two plants remained healthy and free of the pathogen for four years and appeared HLB resistant. Four accessions of *Microcitrus* spp. (two known hybrids) included in the field trial were considered as tolerant plants belonging to category 3. The genus *Microcitrus* is presumed to be monoembryonic and seedling genetic variations may exist. The plants had excellent field tolerance and none appeared to die because of HLB. Symptoms of HLB that typically occur in citrus were not observed in *Microcitrus* spp. In the tolerant accession 'Simmon's trifoliata', two plants died due to HLB and the other six appeared resistant. Psyllids tend to avoid colonizing cultivars of *Poncirus trifoliata*, resulting in plants being exposed to less inoculum (Richardson and Hall 2013; Hall et al. 2015).

Category 4. This group of plants are considered HLB tolerant, since they show recovery from '*Ca. L. asiaticus*' infections. One citrus accession and five citrus relative genera were included in category 4: *Aegle marmelos* (Indian bael), *Aeglopsis chevalieri* (Chevalier's Aeglopsis), *Balsamocitrus dawei* (Uganda Powder flask), *Citrus latipes* ('Khasi' papeda), *Microcitrus australis* (Australian round lime), and *Murraya paniculata* ('Hawaiian mock orange').

Indian bael is a plant of religious significance important to certain ethnic groups. In the field trial, three of the seven Indian bael trees planted died in year 1 due to freeze damage. One plant died in year 2, probably due to HLB, since a Ct value of 27 was recorded. Indian bael had disease symptoms on the branches that tested positive by qPCR. The other four plants had Ct values of 27 to 37 at various times. During year 6, three plants appeared to be free of '*Ca. L. asiaticus*' and one tree had a Ct of 36 for '*Ca. L. asiaticus*'. Five trees of Chevalier's Aeglopsis died during the first winter, presumably because of the cold weather. One surviving plant remained resistant for six years, and the pathogen was never detected from this plant. Two plants had Ct values of 28 in year 2 but, in the subsequent year, the pathogen could not be detected despite extensive sampling and testing. Chevalier's Aeglopsis lacked typical HLB symptoms. However, due to other factors seemingly not related to HLB, both these plants died during year 4 of the study. Uganda powder flask also appeared to recover from infections. Two plants died by year 3, one apparently because of HLB and another died for other reasons. As observed in many other noncitrus genera, the '*Ca. L. asiaticus*'-positive plants of Uganda powder flask did not have identifiable HLB symptoms. In six other plants, Ct values of 28 to 35 were recorded in years 1, 2, and 3; during years 4 and 6, all living plants of this accession were free of '*Ca. L. asiaticus*'. Australian round

lime showed excellent field tolerance similar to other accessions of *Microcitrus* spp. that were evaluated in this field trial. Most of the trees were tolerant and symptom-free in spite of having Ct values of 30 to 38 for '*Ca. L. asiaticus*'. 'Hawaiian mock orange' was considered as an HLB-tolerant accession belonging to category 4. Ct values of 28 to 38 were detected in most plants of 'Hawaiian mock orange' (in six of 28 data points) but, with time, the pathogen was eliminated from the plant. One citrus accession, 'Khasi' papeda, was included in category 4. Four of the eight 'Khasi' papeda plants had '*Ca. L. asiaticus*' Ct values of 24 to 34 during years 1 to 4. However, in year 6, the six surviving plants appeared healthy and had no detectable HLB pathogen, indicating recovery from infections.

Category 5. Only two accessions were grouped under HLB-tolerant category 5, characterized by a delay in '*Ca. L. asiaticus*' infections. Many plants of *Poncirus trifoliata* ('Little-leaf' trifoliata) and *XCitroncirus* hybrid ('X639' trifoliata) had delayed infections. Blotchy mottle leaf symptoms were present in '*Ca. L. asiaticus*'-positive plants in this group.

Category 6. Categories 6, 7, and 8 were all basically susceptible to '*Ca. L. asiaticus*', with typical HLB symptoms. Category 6 consisted of 38 accessions, mostly belonging to the genera *Citrus*, *Severinia*, and *XCitroncirus*. The plants were clearly susceptible (based on '*Ca. L. asiaticus*' titers and disease symptoms) but most of the accessions had all eight plants living for at least four years and the plants retained their leaves, in spite of having disease symptoms and high pathogen titers. Chinese box orange also had classic symptoms of HLB and diseased plants showed stunted growth. HLB in Chinese box orange has been documented earlier (Hung et al. 2001; Hu et al. 2014). In this field trial, among the citrus accessions tested, most of the citrons, limes, and lemons belonged to category 6 and seem to perform better than other citrus types. Certain mandarins, pummelos, and sour oranges also belonged to category 6 (Table 1).

Category 7. In this group, we included accessions that lived for up to four years but had severe disease symptoms including loss of foliage. About 25 accessions, including many mandarins, certain pummelos, sour oranges, some lemons, and limes, were in category 7.

Category 8. Plants in this susceptible group mostly died within the first four years of the field trial. Certain mandarins were grouped under category 8. Table 2 shows the range of '*Ca. L. asiaticus*' Ct values for all accessions during the six-year field trial.

We had eight accessions that could not be grouped under any category because of insufficient data. Table 3 shows Ct values of representative samples from each disease category. Table 4 shows disease categories observed in the different genera included in the field trial.

HLB disease scores associated with ACP abundance and colonization. In a longitudinal study conducted in Fort Pierce, FL, involving the exact same test plants, Westbrook et al. (2011) studied the colonization of ACP. We have compared the HLB categories and '*Ca. L. asiaticus*' titers assigned in the present study with ACP abundance and colonization data (Westbrook et al. 2011) (Table 2). It has been shown that native rutaceous plants that serve as hosts for the psyllid can affect the epidemiology of HLB and noninfection of certain taxa may be due to the inability of the psyllid to transmit the HLB pathogen (Sétamou et al. 2016).

Plants in category 1 had either medium or low scores of ACP infestation. In category 2, *Casimiroa edulis* (White sapote) was reported as a nonhost for ACP, with a score of 0 for both adult and nymph infestations (Halbert and Manjunath 2004; Westbrook et al. 2011). However, we have detected a transient presence of '*Ca. L. asiaticus*', indicating that occasional psyllid feeding may occur. *Zanthoxylum ailanthoides* (Japanese prickly ash) had a low score of 0.29 for adult ACP infestations and did not support colonization (Westbrook et al. 2011). Most species of genus *Zanthoxylum* are reported to be poor hosts for the ACP (Halbert and Manjunath 2004). The Australian accession *Eremocitrus glauca* (Australian desert lime) was not a preferred host for the psyllid, since it had low scores of 0.2 and 0.9 for adult and nymph infestations, respectively (Westbrook et al. 2011). In contrast, *Murraya paniculata* ('Orange jasmine') and

Bergera koenigii (curry leaf) are known to be preferred hosts for ACP (Damsteegt et al. 2010; Tsai et al. 2002; Westbrook et al. 2011).

In category 3, with variations observed in seedling populations, *Poncirus trifoliata* ('Simmon's trifoliata') had very poor scores for psyllid infestation and colonization (Westbrook et al. 2011). Other accessions in this category had psyllid infestation numbers ranging from 0.14 in Large-leaf Australian lime to 0.27 in 'Faustrimedon'. Colonization numbers ranged from 0.48 in Australian finger lime to 1.68 in 'Sydney hybrid'. In general, psyllid colonization levels were similar to other HLB-sensitive accessions. Most accessions that we grouped in category 4 had moderate psyllid ratings. The score ranged from 0.27 to 0.70 for adults (in Indian bael and 'Hawaiian mock orange', respectively) and 0.5 to 1.77 for nymphs. 'Hawaiian mock orange', despite being favorable for psyllid colonization, still appears to have tolerance to HLB. In category 5, consisting of HLB tolerant plants showing delayed '*Ca. L. asiaticus*' infections, only two accessions were included. 'Little-leaf' trifoliata is not a

Table 3. Representative taxa from each of the eight disease categories and yearly levels of the huanglongbing (HLB) pathogen^a

Name of the taxa (CRC number)	Year 1	Year 2	Year 3	Year 4	Year 6
Category 1 (immune: no pathogen detected)					
<i>Glycosmis pentaphylla</i>	40	40	40	40	40
Orange berry (3285)	40	40	40	40	40
	40	40	40	40	40
	40	40	40	40	40
Category 2 (resistant: transient replication)					
<i>Eremocitrus glauca</i> hybrid	38	40	40	40	36
Australian desert lime (4105)	39	40	40	40	D*
	36	35	40	40	40
	40	40	40	40	40
Category 3 (tolerant: seedling variation)					
<i>Microcitrus australasica</i>	30	40	36	31	30
Australian finger lime (1484)	36	40	40	40	40
	40	35	40	40	40
	40	40	40	40	35
Category 4 (tolerant: recovery from infection)					
<i>Balsamocitrus dawsonii</i>	40	30	40	40	40
Uganda powder flask (3514)	40	33	40	40	40
	28	30	D*		
	40	35	40	40	40
Category 5 (tolerant: delayed infections)					
<i>Poncirus trifoliata</i>	40	40	26	28	30
var. Little-leaf (4007)	37	40	40	40	34
	40	38	30	30	29
	40	40	30	28	29
Category 6 (susceptible, retained leaves)					
<i>Citrus medica</i>	24	32	27	29	30
'Diamante citron (3523)	27	26	31	29	33
	28	26	28	30	30
	24	29	29	29	28
Category 7 (susceptible, died after four years)					
<i>Citrus sinensis</i>	40	28	23	23	D**
Pineapple sweet orange (3858)	40	31	26	26	D**
	40	25	25	D**	
	24	26	D**		
Category 8 (susceptible, died in two years)					
<i>Citrus reticulata</i>	29	D**			
Unnamed mandarin (3812)	35	24	D**		
	40	25	D**		
	31	22	D**		

^a As estimated by real time polymerase chain reaction results shown as cycle threshold (Ct) values. Ct values show an inverse relationship to the level of the pathogen. A value of 40 indicates no detectable pathogen. Decreasing Ct values indicate increasing levels of the pathogen. D* indicates death due to environmental factors; D** represents death due to HLB.

preferred host for ACP (Westbrook et al. 2011), thus, tolerance in this cultivar to HLB may be partially attributed to the psyllid preference factor. The second tolerant accession, a *Citrus* × *Poncirus* hybrid ('X639' trifoliata hybrid) had moderate scores for adult and nymph infestation levels. The susceptible accessions in categories 6, 7, and 8 had adult infestation values ranging from 0.25 ('Koji' mandarin) to 1.11 ('Tien Chieh' mandarin); nymph infestation scores ranged from 0.48 (in Chinese box orange) to 2.09 (in 'Swingle' citrumelo).

Discussion

In the present study, we have evaluated the disease response of 65 citrus accessions and 33 accessions from 20 citrus-related genera when exposed to natural transmission conditions. As a systematic study using cataloged accessions, we believe that the results obtained will be valuable to validate the HLB host plant lists, to formulate regulations aimed toward disease control, to alleviate HLB epidemics in regions where the disease is not yet widespread, and—most importantly—to identify tolerant and resistant cultivars of *Citrus* and other related genera. The resistant plants will be useful in the future to understand mode of resistance and to characterize genes associated with resistance. This information may be utilized to generate resistant commercial cultivars, either by conventional breeding or by transgenic approaches. The citrus accessions chosen for the study are presumed to represent the genetic diversity in the genus *Citrus* from the collection maintained at Riverside, CA (Barkley 2003). The field trial was conducted in the HLB-endemic region of southeastern Florida, where a high psyllid population has established naturally. The type of rootstocks used for citrus cultivation are known to have an effect on HLB disease incidence in the scion cultivars (Albrecht and

Table 4. Huanglongbing disease categories in different genera tested in this study^a

No.	Genus (accessions tested)	Taxonomical grouping	Disease category
1	<i>Casimiroa</i> (1)	Toddalioidae	2
2	<i>Zanthoxylum</i> (1)	Toddalioidae	2
3	<i>Bergera</i> sp. (1)	Aur/Clauseneae/ Clauseninae	2
4	<i>Clausena</i> (2)	Aur/Clauseneae/ Clauseninae	1 and *
5	<i>Glycosmis</i> (1)	Aur/Clauseneae/ Clauseninae	1
6	<i>Murraya</i> (2)	Aur/Clauseneae/ Clauseninae	2 and 4
7	<i>Aegle</i> (1)	Aur/Citreae/ Balsamocitrinae	4
8	<i>Aeglopsis</i> (1)	Aur/Citreae/ Balsamocitrinae	4
9	<i>Afraegle</i> (1)	Aur/Citreae/ Balsamocitrinae	*
10	<i>Balsamocitrus</i> (1)	Aur/Citreae/ Balsamocitrinae	4
11	<i>Atalantia</i> (1)	Aur/Citreae/Citrinae	*
12	<i>Citropsis</i> (1)	Aur/Citreae/Citrinae	*
13	<i>Eremocitrus</i> (1)	Aur/Citreae/Citrinae	2
14	<i>Fortunella</i> (1)	Aur/Citreae/Citrinae	*
15	<i>Microcitrus</i> (4)	Aur/Citreae/Citrinae	3 and 4 (2 accessions each)
16	<i>Naringi</i> (1)	Aur/Citreae/Citrinae	2*
17	<i>Poncirus</i> (2)	Aur/Citreae/Citrinae	3 and 5
18	<i>Severinia</i> (5)	Aur/Citreae/Citrinae	6 and *
19	<i>XCitroncirus</i> (4)	Aur/Citreae/Citrinae	5 (1 accession); 6 (3 accession)
20	<i>XMicrocitronella</i> (1)	Aur/Citreae/Citrinae	3*
21	<i>Citrus</i> (65)	Aur/Citreae/Citrinae	3 (1 accession) 4 (1 accession) 6 (32 accessions) 7 (25 accessions) 8 (6 accessions)

^a Aur = Aurantioideae; asterisks (*) indicate insufficient data.

Bowman 2012; Shokrollah et al. 2011). Since the test plants in this field trial were grown as seedling trees without any rootstock, we were able to evaluate the cultivars without the confounding effects of rootstocks. Sample collections were conducted during October or November, since the bacterial titer is known to be highest during these periods in Florida (Manjunath et al. 2008; Wang et al. 2006).

In the current field trial, the experimental seedlings were raised from open-pollinated seeds collected from trees growing in a grove with multiple varieties from the subfamily Aurantioideae. Since the seed parent is known, the eight seedlings may represent half-sib families. If the seed parent is monoembryonic, the eight plants tested are likely to be genetically different from each other, each being a unique hybrid with possibly different pollen parents. Since some citrus is self-compatible, it is also possible that seedlings of some accessions result from self-pollination. If the seed parent has a high degree of nucellar embryony, it is likely that the eight seedlings are clones of the seed parent and represent replicate plants. In accessions with nucellar embryony, certain seedlings may be from zygotic embryos, while others may be nucellar clones of the seed parent (Koltunow et al. 1996). Nucellar seedlings do not develop if pollination does not occur, since the endosperm fails, and its extent is cultivar-dependent (Altaf et al. 2001; Khan and Roose 1988; Webber 1930; Wright 1937). Information on the occurrence of nucellar embryony in many accessions included in this study is not available. The percentage of nucellar embryos will vary depending on the pollen parent, type of pollinator, and environmental factors (Mondal and Saha 2014). Generally, citrons and pummelos do not have nucellar embryony (Kishore et al. 2012). Many mandarins, sour oranges, sweet oranges, lemons, limes, grapefruit, and trifoliate are known to possess nucellar embryony (Nakano et al. 2012; Soost and Roose 1996). Among the citrus relatives included in the study, Indian bael has been shown to have nucellar embryony (Johri and Ahuja 1957). Curry leaf plant has been reported to have polyembryony, which predicts the occurrence of nucellar embryony (Madalageri and Gowda 1987). Table 2 indicates accessions in which the nucellar embryony or monoembryony status is known. Certain accessions are presumed to have monoembryony or nucellar embryony based on group characters. Even when the nucellar embryony status of a cultivar is known, it is difficult to speculate if the eight seedlings of an accession used in our trials arose as nucellar seedlings or if they are zygotic seedlings. In one of the categories we identified, the HLB-tolerant plants appear to show seedling variations (category 3). Out of the six accessions in this category, three are presumed to be monoembryonic (Australian finger lime, 'Sydney hybrid', and Large-leaf Australian wild lime) and one accession is probably polyembryonic ('Simmon's trifoliolate'); the status of 'Mountain' citron and 'Faustrimedin' is not known. The polyembryonic 'Simmon's trifoliolate' probably has seedlings of different genotypes, since we clearly saw that some seedlings were susceptible while others were tolerant to HLB. In *Microcitrus* spp., nucellar embryony has not been reported and we assumed that the accessions are monoembryonic. Two types of seedlings (susceptible and tolerant) seem to be present in the progeny from *Microcitrus* spp. and citrus hybrids with *Microcitrus* parentage.

Plants can develop innate immunity because of structural, chemical, or protein-based defenses (Doughari 2015). In the present study, only two accessions (from a total of 90) are totally immune to the HLB pathogen (Table 1). The genus *Clausena* has several species that are commonly grown in China and South East Asia. Classical HLB symptoms have been described in *Clausena lansium* Skeels growing in the Guangdong province of China (Deng et al. 2007). The infected tree in China was reported to have yellow shoots typical of HLB. However, only very low titers of the pathogen were recorded and '*Ca. L. asiaticus*' could only be detected by nested PCR (Deng et al. 2007; Ding et al. 2005). The cataloged accession of *Clausena excavata* was found to be immune to the Florida '*Ca. L. asiaticus*' strain in the current study.

Resistance to disease is invaluable for the successful management of citrus HLB. Six accessions in the study were considered as HLB-resistant hosts (category 2). Unlike the immune accessions, the resistant taxa were capable of supporting multiplication of the pathogen

transiently to various levels, as shown in this study. Although the pathogen was present for only a short duration, it is possible for a psyllid feeding on such a plant to acquire the pathogen and transmit it to a more receptive host plant, such as *Citrus* spp. (Lee et al. 2015). Our data clearly showed that the pathogen always disappeared in the resistant types and did not colonize the plant host. From a regulatory point of view, plants categorized as resistant in our study may need to be included in the list of regulated plants, especially in regions where HLB is in the initial stages of the imminent epidemic, to avoid disease spread via alternate plant hosts.

White sapote is not a preferred host for the psyllid (Halbert and Manjunath 2004; Westbrook et al. 2011). Since this plant is grown in California by rare fruit growers and horticulturists, mainly in regions where citrus is cultivated, and it may act as an alternate host for HLB, regulatory precautions are warranted. Greening-associated '*Ca. L. asiaticus*' or disease symptoms have never been reported from curry leaf. Hung et al. (2000) extracted DNA from greenhouse-grown curry leaf plants that were exposed to HLB and hybridized to '*Ca. L. asiaticus*' nucleic acid. '*Ca. L. asiaticus*' DNA was not detected in these experiments, indicating absence of '*Ca. L. asiaticus*' in curry leaf (Hung et al. 2000). Transmission of HLB from citrus to curry leaf has not been successful under greenhouse conditions (Damsteegt et al. 2010; Hung et al. 2000). Conflicting reports exist regarding the susceptibility or resistance of *Murraya paniculata* to citrus HLB (Hung et al. 2000, 2001; Lopes et al. 2010). In the current field trial, the 'Orange jasmine' trees were very healthy-looking, while 'Hawaiian mock orange' showed some chlorotic branches. In other studies, yellow shoots and insignificant blotchy mottle have been reported from *Murraya paniculata* (Nguyen 2012). Since curry leaf and 'Orange jasmine' are preferred hosts of ACP, the chances of pathogen spread (liberibacter that may be transiently present in the plant) through psyllids would be potentially high. Large ACP populations have been recorded from urban plantings of *M. paniculata* in Florida (Hall and Rohrig 2015). Transmission experiments showed that the pathogen could be transmitted experimentally from 'Orange jasmine' to citrus (Damsteegt et al. 2010; Tirtawidjaja 1981). The Asian greening-associated pathogens in Taiwan do not multiply either in *Murraya paniculata* or in *Bergera* spp. (Hung et al. 2000; Toorawa 1998). Surveys by Walter et al. (2012a and b) of *M. paniculata* plantings in Florida indicated that the incidence of the HLB-associated pathogen was extremely low, both in the plants and in ACP infesting the plants.

We used the '*Ca. L. asiaticus*' Ct values to determine and define the disease response categories (Table 1). Some of the samples with high Ct value may actually be negative for '*Ca. L. asiaticus*'. Since one molecule of target DNA under ideal conditions of real time PCR should give a Ct value of about 37 (G. McCollum, *personal communication*), DNA deposited by psyllids while feeding or the presence of dead bacteria may result in a positive Ct value (>37 and <40), even if there is no replication. It has been demonstrated that acquisition of '*Ca. L. asiaticus*' is more efficient when a psyllid nymph feeds on an infected plant than when an adult psyllid feeds (Inoue et al. 2009; Pelz-Stelinski et al. 2010). Based on this criterion, *Zanthoxylum* spp. may not be an alternate host for HLB, since the psyllid does not colonize on this accession.

Australian desert lime (*Eremocitrus glauca* hybrid) was highly resistant to '*Ca. L. asiaticus*' in our trials, however, there are no published reports on the HLB host status for *Eremocitrus* spp. It is likely that since '*Ca. L. asiaticus*' has never encountered this host in its natural habitat in Australia, it may lack adaptation to infect Australian desert lime. Ongoing studies with both *Eremocitrus glauca* (Citrus Research Center number CRC 4107) and some of the available natural hybrids of *E. glauca* (with citrus) have confirmed both the existence of a high level of resistance in *E. glauca* as well as the inheritance of resistance in hybrid accessions (data not shown).

In category 3, seedling variations in plants within an accession resulted in two different types of responses to HLB challenge—susceptibility to the disease in some seedling trees and a high degree of resistance in some other seedlings. This may arise due to the genetic variability in the open-pollinated seeds or due to segregation of traits in hybrid

accessions. Some seedlings of 'Sydney hybrid' showed a high titer of 'Ca. L. asiaticus' and severe stunting, while others showed normal growth and no detectable 'Ca. L. asiaticus'. Three species of genus *Microcitrus* and two hybrids having *Microcitrus* parentage were evaluated in the study. Genus *Microcitrus* is presumed to be monoembryonic and the seedlings are likely to be genetically different from each other. In spite of having a high 'Ca. L. asiaticus' titer (Ct values of 24 to 30 recorded in many), plants with *Microcitrus* parentage displayed good field tolerance. Typical HLB symptoms are not seen on Australian citrus types. The die-back of the shoots observed in *Microcitrus* spp. may be associated with HLB infection. Psyllids can feed and colonize well on all of the *Microcitrus* accessions included in the study. It may be prudent to include them on the exclusion list for HLB quarantine purposes.

'Simmon's trifoliolate' showed resistance to HLB in five out of eight seedlings. Since this accession may have polyembryony, it may be interesting to analyze resistant and susceptible seedlings. Trifoliolates have been shown to have more resistance to HLB than other citrus groups (Albrecht and Bowman 2011, 2012; Boava et al. 2015; Folimonova et al. 2009). Six accessions with trifoliolate parentage were included in the current study. Among the *Poncirus trifoliata* accessions, 'Simmon's trifoliolate' was HLB tolerant (category 3); 'Little-leaf' trifoliolate was more susceptible (category 5), in spite of the psyllid abundance and colonization scores being low (Aubert 1987; 1990; Richardson and Hall 2013; Westbrook et al. 2011). In addition to effects on psyllid feeding behavior, antibacterial compounds associated with the *Poncirus trifoliata* parentage may be responsible for disease tolerance (Albrecht and Bowman 2011). The two *Poncirus* accessions studied are resistant to ACP (Westbrook et al. 2011) and tolerant to HLB. Interestingly, HLB tolerance recorded in *Poncirus trifoliata* accessions was not observed in four hybrids of *Citrus* and *Poncirus* tested in this study, 'Rusk' citrange, 'S-281' citrangelo, 'Swingle' citrumelo (*C. paradisi* Duncan × *P. trifoliata*), and 'X639' (*Citrus reshni* Cleopatra mandarin × *Poncirus trifoliata*).

Even though, we have categorized many accessions as resistant or highly tolerant and placed them in categories 2 and 3, many of them support transient replication of 'Ca. L. asiaticus' to varying levels. While these accessions may have utility in breeding for disease resistance, they should be considered as potential alternate hosts for regulatory purposes, especially in regions like California, where the disease has not yet established. In the United States, prior to 2008, genus *Murraya* was not regulated and there was free movement of ornamental *Murraya* spp. throughout the state of Florida (Halbert et al. 2012). Based on a survey conducted during 2005 and 2006, we reported 'Ca. L. asiaticus'-positive nymphs (with Ct values as low as 22) collected from ornamental *Murraya* plants marked for sale in big box stores located in Nassau county in the northern part of Florida, where commercial citrus was not grown (Manjunath et al. 2008). Positive psyllid nymphs indicate presence of the pathogen in the plant on which the nymphs are feeding. The origin of these *Murraya* plants was traced to Miami Dade County, where HLB was reported at that time. Tens of thousands of potted *Murraya* plants were exported out of the Miami Dade area during this period (Halbert et al. 2012). It is likely that unregulated movement of *Murraya* spp. during the early stages of the epidemic might have contributed toward widespread dispersal of the disease in Florida (Manjunath et al. 2008). Similar observations were made by Lopes et al. (2010) regarding the spread of HLB through the movement of *Murraya* plants in Brazil. Transmission of HLB from *Murraya* plants to *Citrus* plants has been demonstrated (Damsteegt et al. 2010; Zhou et al. 2007). The disease response of different *Murraya* cultivars to various liberibacter isolates are known to vary (Lopes et al. 2010; Tirtawidjaja 1981). In this study, the two accessions of *Murraya* spp. showed resistant and tolerant responses to the pathogen in the field. The low 'Ca. L. asiaticus' titers we observed in *Murraya* spp. agrees with similar results reported by other researchers (Damsteegt et al. 2010; Walter et al. 2012a and b).

The Australian limes (genera *Eremocitrus* and *Microcitrus*) are close relatives of citrus but are morphologically distinct and are presumed to have diverged from genus *Citrus* about 3.5 million years

ago (Schwartz et al. 2015). According to some taxonomists, they are grouped under the genus *Citrus* (Mabberley 1997). In the subtribe Citrinae, five of the six genera (*Citrus*, *Poncirus*, *Fortunella*, *Microcitrus*, and *Eremocitrus*) are known to be sexually compatible with each other (Iwamasa et al. 1988). The high degree of resistance found during this study in genus *Poncirus* and the two Australian genera *Eremocitrus* and *Microcitrus* is probably of great value, since they can be crossed with citrus. It may be possible to develop HLB-resistant hybrid rootstocks and scions using these as parents in breeding trials. Hybrid populations may also serve as excellent resources for marker analysis and biochemical and molecular characterization of resistance mechanisms. Other sexually noncompatible members of Aurantioideae may have different mechanisms of resistance and may be utilized for HLB mitigation. Certain graft-compatible accessions may be able to provide good resistance if used as approach grafts. Planting citrus with two rootstocks, while expensive, may be worthwhile as an interim approach, if resistance to HLB can be obtained.

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