Apparent Tolerance to Huanglongbing in *Citrus* and *Citrus*-related Germplasm

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Abstract. In a Fort Pierce, FL, field planting, plant growth, and Huanglongbing (HLB) severity were assessed as indicators of HLB tolerance on progenies of 83 seed-source accessions of *Citrus* and *Citrus* relatives mainly from the Riverside, CA, genebank. The HLB-associated pathogen [*Candidatus Liberibacter asiaticus* (CLas)] and vector [asian citrus psyllid (ACP), *Diaphorina citri*] were abundant, and trees were naturally challenged for 6 years before metrics (leaf mottle, percent canopy mottle, overall health, canopy density, canopy width, canopy height, and trunk diameter) were collected in Oct. and Nov. 2015. The healthiest trees with low or no HLB symptoms were distant citrus relatives: *Balsamocitrus dawei*, *Bergera koenigii*, *Casimiroa edulis*, *Clausena excavata*, *Muraya paniculata*, and one accession of *Severinia buxifolia*. Within *Citrus*, most of the healthiest trees with densest canopies, little leaf loss, and greater growth were those with pedigrees that included *Citrus medica* (citron). These included progenies of *Citrus* hybrid (*‘Limon Real’*), *Citrus limetta*, *Citrus limioloides*, *Citrus limonia*, *C. medica*, *Citrus volkameriana*, and some *Citrus limon* accessions. Trees in this category exhibited distinct leaf-mottle characteristic of HLB and substantial pathogen titers, but maintained dense canopies and exhibited good growth. Trees from seed-source accessions in the genotype *Citrus* without citron in their background were generally among the least healthy overall with less dense canopies. The exceptions were progenies of two *Citrus aurantium* accessions, which were markedly healthier than progenies of other *Citrus* seed-source accessions not derived from citron. Linear regression analysis, between metrics collected and pedigree of seed parent, indicated that percentage of citron in the pedigree significantly correlated with measures of tolerance. Although no commercial *Citrus* genotypes yielded progenies with strong HLB resistance, in this field experiment several progenies maintained dense canopies and good growth, and may be useful for breeding HLB tolerant cultivars.

HLB is a highly destructive disease of *Citrus* associated with the fastidious gram-negative, obligate-parasite, psloem-limited α-Proteobacteria, *Candidatus Liberibacter sp.* In Florida, HLB is associated with CLas, and is vectored by the ACP, *Diaphorina citri* (Bove, 2006; da Graça and Korsten, 2004; Garnier and Bove, 1983; Gottwald, 2010; Halbert and Manjunath, 2004; Hall et al., 2013; Jagoueix et al., 1994). To date, CLas has not been cultured to purity, although mixed cultures of this bacterium have been reported (Davis et al., 2008; Scheller et al., 2009). The first likely description of HLB was published in India in 1927, and symptoms were attributed to psyllid damage (Husain and Nath, 1927). However, the first rigorous scientific description of HLB was not until 1938 (Chen, 1943). ACP was first detected in Florida in 1998 and the first confirmation of HLB was in 2005 (Halbert, 2005); followed by Louisiana in 2008, South Carolina and Georgia in 2009; and Texas and California in 2012 (Kumagai et al., 2012; Kunta et al., 2012). HLB is an immediate threat to the financial survival of the Florida citrus industry, and also is threatening other citrus-growing regions in the United States and worldwide. Presently, greater than 80% of Florida citrus trees are infected with CLas (Singerman and Useche, 2015) with the great majority exhibiting associated disease symptoms: yellow asymmetrical patterns (blotchy mottle) on leaves, vein yellowing and thickening, thinning canopies, dieback, increased fruit drop, and a measurable decline in fruit quality. At the anatomical level, HLB induces progressive degeneration of the phloem tissue resulting in partial or complete collapse of the phloem with concomitant callose plugging of lateral pits and sieve plates (Achor et al., 2010; Chen et al., 2010; Folimonova and Achor, 2010; Koh et al., 2012; Schneider, 1968). In roughly a decade, the Florida citrus industry has experienced a decline in crop production of more than 50% (USDA National Agricultural Statistics Service, 2015). The U.S. citrus industry, as with the rest of the world, primarily relies on cultivars with a very narrow genetic base. The most important commercial cultivars, excluding lemons and limes, are the product of only two parental species: *Citrus reticulata* and *Citrus maxima*. Additionally, sweet orange, which is among the most broadly cultivated tree fruits in the world, possesses little genetic variation despite having a diverse array of desirable phenotypic traits (Curt et al., 2016; Gulsen and Roose, 2001; Matsumoto and Magano, 2013; Novelli et al., 2006). With the world’s citrus production at risk of collapse, identifying resistance and tolerance to HLB is important to understanding this pathosystem, and cultivars that tolerate HLB are needed to sustain the citrus industry.

Resilience in citrus relatives has only been observed in genera not widely classified as *Citrus* (Ramadugu et al., 2016), which may offer valuable genetic diversity for tolerance to HLB as well as other *Citrus* diseases. Wild *Citrus* species are increasingly threatened worldwide and it is crucial to make efforts to conserve these resources, which may be vitally important for future *Citrus* cultivar improvement (Malik et al., 2013). Though true resistance to HLB is the ultimate goal, tolerance to HLB may be invaluable for short-term survival of the citrus industry both here in the United States and abroad. A number of HLB-tolerant cultivars have been reported by research groups around the world (Koizumi et al., 1993; Ramadugu et al., 2016; Shokrollah et al., 2009; Stover and McCollum, 2011; Stover et al., 2014). Knowledge of the genetics underlying resistance and tolerance to HLB, from both *Citrus* and *Citrus* relatives, may be useful for conventional and biotechnology-based breeding. Further work is needed to assess this potential and to realize its commercial value by mobilizing resistance and tolerance into the range of commercial fruit types necessary to satisfy consumer demands. The objective of this study was to evaluate growth and visible disease severity metrics on progenies of 83 diverse seed-source accessions of *Citrus* and *Citrus* relatives in a Florida field planting to identify their relative tolerance to HLB.
Materials and Methods

**Plant source materials and experimental design.** Seeds, representing a broad cross-section of diversity in *Citrus* and *Citrus* relatives, were acquired from the USDA-ARS National Clonal Germplasm Repository for *Citrus* and Dates at the University of California (UCR), Riverside, CA (http://www.citrusvariety.ucr.edu). Seeds used for this study were collected from 124 accessions of *Citrus* and *Citrus* relatives, mainly of the subfamily Aurantioideae, family Rutaceae, representing ≈85% of the genetic diversity within the UCR collection (Barkley, 2003). Good germination was obtained from about 100 accessions, generating ≈886 seedlings. Information on the phylogenetic relationships of the seed-source genotypes used for this study, are described in Barkley (2003) and Bayer et al. (2009). Seeds of *Zanthoxylum aianthoideum* (subfamily Toddaliaeae) were secured from the University of Georgia. Seeds of *Casimiroa edulis* (subfamily Toddaliaeae), *Afroega paniculata* and *Aegle marmelos* (subfamily Aurantioideae) were obtained from the Fruit and Spice Park (Miami-Dade County, FL).

Progeny growth and development, field planting, and general care and maintenance were described in Richardson et al. (2011) and Westbrook et al. (2011) and were consistent with citrus production practices for processing before HLB. Briefly, seeds were germinated in individual plastic cells (Containers™, Stuewe and Sons Inc., Tangent, OR) and grown under greenhouse conditions at the USDA-ARS U.S. Horticultural Research Laboratory in Fort Pierce, FL. Six-to-nine-month-old seedlings, eight single-plant replicates for each seed parent, were planted at the USDA-ARS, Fort Pierce, FL, farm during June and July 2009. A randomized complete block design was used with 0.6 m spacing within rows and there was 3.5 m between rows. The planting was irrigated regularly using micro sprinklers and fertilized using a regime similar to that of a new commercial planting of *Citrus*. To maintain high ACP pressure and maximize challenge by CLas, no insecticides were applied, and high ACP populations were apparent year round.

**Growth measurements.** Tree height, canopy width, tree canopy volume (TCV), trunk diameter, canopy density, overall health, and HLB phenotypic leaf symptoms (mottle, and mottle) were assessed during 2 weeks from planting to flowering. Tree volume was calculated using the model CMR-25; Mound City, IL), and tree canopy width was measured on an east/west and north/south plane. TCV was calculated on the assumption that tree shape was that of a half sphere (TCV = 4πA/6 x H x W1 x W2). Tree diameter was measured 10 cm above the soil line.

**Disease severity assessment.** For scoring of disease severity metrics, a five-point scale was used where 5 is worst and 1 is one is fully healthy (devoid of visible disease symptoms), at a half point resolution. Subjective assessments of trees were conducted by two people to reach a consensus score.

Scoring for leaf mottle was based on the presence of yellow asymmetrical patterns (blotchy mottle) using the following scale: 1 = no visible degree of mottling; 2 = light leaf mottling; 3 = moderate level of leaf mottling; 4 = heavy leaf mottling; 5 = severe leaf mottling. The percentage of the entire canopy leaf surface mottled on each tree was also estimated. Leaves affected by other visible stresses causing leaf yellowing (e.g., canker and leaf miners) were excluded. Canopy density was based on visually inspecting the tree canopy from all quadrants to determine an overall score. Scoring was as follows: 1 = completely healthy, dense canopy; 2 = light canopy thinning; 3 = moderate canopy thinning; 4 = heavy canopy thinning; 5 = worst case canopy thinning—most branches devoid of leaves. Scoring for dieback was determined as follows: 1 = completely healthy, no observed dieback; 2 = a single medium-to-large branch with some level of dieback; 3 = multiple small, medium or large branches, three to four branches total, exhibiting some level of dieback; 4 = heavy dieback, four to five branches of medium to large size showing some level of dieback; 5 = worst case trees with five or more branches (medium-to-large) with dieback. The scoring for overall health was as follows: 1 = completely healthy, no signs of disease; 2 = very good health; 3 = moderate/average health; 4 = poor health; 5 = dead or very dead, tree crown thinning, many of which were near death.

**Statistical analysis.** Means and standard deviations were calculated using Excel (version 2013; Microsoft, Redmond, WA). Regression analyses of several data sets were conducted (also with Excel) to assess relationships between measures of HLB tolerance and estimated pedigree percentages for each seed parent (pedigrees were not determined for individual seedlings) from each of the progenitor species that gave rise to all cultivated citrus: citron (*C. medica*), pummelo (*C. maxima*), mandarin (*C. reticulata*), and papada (*C. micrantha* or related species). The pedigree estimates vary in confidence with some based on recently published genomic sequence analyses (Curk et al., 2016) and others on published speculation based on morphology (Hodgson, 1967). Relationships are indicated as positive when health increases with increasing percentage of the indicated progenitor species in the seed-source accession pedigree.

**Results and Discussion**

In this study, growth and disease severity metrics were evaluated on progenies of 83 diverse seed-source progeny of *Citrus* and *Citrus* relatives in a Florida field planting to identify the relative tolerance to HLB under natural conditions. This field trial was established in 2009 and conducted in an HLB-endemic region with year-round ACP pressure. Data were collected over 2 weeks in the autumn (from late Oct. through Nov. 2015), when CLas titer and symptoms are the highest in Florida (Manjunath et al., 2008; Wang et al., 2006). Initial infection with CLas and development of HLB symptoms is not uniform. After 6 years, the interaction between the pathogen and each host tree is well established with only gradual subsequent changes, and the effects on growth essentially reflect an integration of the disease response over the planting life. Therefore, the data we collected at this single time point is ideal for assessing the cumulative response to HLB. For this present study, CLas titer data from Ramadugu et al. (2016) are provided as a reference, and the 2015 data CLas titer data were collected within 2 weeks of our data collection.

Nucellar embryony varies significantly within the Rutaceae (Frost and Soost, 1968); consequently, the progenies employed for this study were either genetically identical to the maternal parent (seeds serving as clonal propagules) or half-sib hybrids with only the seed parent known. Therefore, only the seed parent is known for this material and all discussions are based on the seed-source accessions for each tree in the study. For progeny of each seed-source accession, growth measurements (TCV and trunk diameter), and disease severity metrics (leaf mottle, percent canopy mottle, overall health, canopy density, and dieback) are summarized in Table 1 along with accession pedigrees and CLas titer levels as Ct from qPCR (5-year mean and levels for 2015) from Ramadugu et al. (2016). It is important to note that Ct is inversely related to CLas titer. Data on fruit were not collected because only a small proportion of trees were cropping and in these young trees, differences were influenced by the relative precocity of the genotypes tested.

Progeny of seed-source accessions outside the genus *Citrus* were among the healthiest and many of these have been shown to have substantial resistance to HLB with little or no CLas detectable (Ramadugu et al., 2016; Table 1). Across all metrics, except for leaf-mottle severity and percent mottle, progeny of many *Citrus* seed-source accessions with citron in their pedigree outperformed other *Citrus*.

**Overall health.** *Citrus* with citron in their pedigree generally had an overall health score similar to trees outside the genus *Citrus*, both near moderate/average, whereas *Citrus* without citron in their pedigree generally scored a rating of poor (Table 1). The healthiest progeny group for *Citrus* with citron in the pedigree was from ‘Volkamer’ lemon hybrid (CRC 3050) with a mean of 2.2 (very good/good health). However, this overall health score was similar to that of ‘Olve- lands’ sour orange (CRC 2717) progeny, the healthiest *Citrus* without citron in pedigree, with an overall health score of 2.3 (very good/good health) (Table 1). In a recent publication looking at HLB and bacterial titer levels for these same trees over 6 years (Ramadugu et al., 2016), it was shown that all trees in the genus *Citrus* had substantial
Table 1. Growth and disease severity metrics for trees of each of 83 seedling populations from diverse seed-source accessions from the National Clonal Germplasm Repository for Citrus and Dates. Trees were evaluated at the USDA Fort Pierce, FL, farm over 2 weeks late October through Nov. 2015, 6 years after planting in an area with severe huanglongbing and natural challenge with Asian citrus psyllid. Overall health, canopy density, dieback, and leaf-mottle severity were scored using a five-point scale, where five was the worst and one was fully healthy. Other metrics were percent blotchy mottle (% of canopy area), tree canopy volume, and trunk diameter. For each seed-source accession, pedigree estimates are indicated from the four progenitor Citrus species (contributing to development of most citrus scion cultivars) and other genera."
| Common name of seed parent | (CRC accession no.) | Overall health (1-5 scale) | Canopy density (1-5 scale) | Dieback (1-5 scale) | Leaf mottle (1-5 scale) | Leaf canker (1-5 scale) | Leaf spot (1-5 scale) | Trunk dieback (1-5 scale) | Tree survival (n=8) | 2015 Cts | 2015 Cts | Pedigree* |
|---------------------------|---------------------|--------------------------|--------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-----------|-----------|----------|
| 'Mexican lime' (3822)     | (0.76)              | (0.87)                   | (0.58)                   | (0.04)            | (38.2)            | (40.02)           | (41.16)          | (4.98)            | (4.11)            | 328.1     | 28.6      | 50       |
| 'Rusk citrange trifoliate hybrid (301) | (0.46)              | (0.37)                   | (0.27)                   | (10.77)           | (6.46)            | (2.77)            | (1.71)           | (4.42)            | (2.05)            | 37.5      | 12.5      | 50       |
| 'Indian' citron hybrid (661) | (0.53)              | (0.69)                   | (0.89)                   | (9.2)             | (10.50)           | (1.15)            | (4.42)           | (2.05)            | (2.05)            | 37.5      | 12.5      | 50       |
| 'Alemow' papeda (3842)    | (0.26)              | (0.59)                   | (0.83)                   | (16.0)            | (10.19)           | (2.97)            | (1.71)           | (4.42)            | (2.05)            | 37.5      | 12.5      | 50       |
| 'Orange berry' 'Gin berry' (3285) | (0.89)              | (0.93)                   | (0.96)                   | (0.00)            | (13.21)           | (4.67)            | (1.71)           | (4.42)            | (2.05)            | 37.5      | 12.5      | 50       |
| 'Little-leaf' trifoliate (4007) | (0.96)              | (1.26)                   | (1.09)                   | (0.71)            | (10.79)           | (1.86)            | (4.67)           | (1.71)            | (4.42)            | 37.5      | 12.5      | 50       |
| 'Sydney hybrid' (1485)    |                      |                          |                          |                   |                   |                   |                   |                   |                   | 37.5      | 12.5      | 50       |
| 'Iranian' lemon (3885)    | (0.41)              | (0.82)                   | (0.86)                   | (0.45)            | (16.9)            | (25.17)           | (1.85)           | (3.10)            | (2.55)            | 37.5      | 12.5      | 50       |
| 'Nasnaran' mandarin (2485) | (0.38)              | (0.50)                   | (0.63)                   | (0.69)            | (21.5)            | (7.16)            | (1.54)           | (4.63)            | (1.96)            | 37.5      | 12.5      | 50       |
| 'Zhuhai' sour orange hybrid (3930) | (1.08)              | (1.10)                   | (0.96)                   | (0.22)            | (29.2)            | (8.77)            | (1.70)           | (4.67)            | (1.96)            | 37.5      | 12.5      | 50       |
| 'Swingle citrumelo' (3771) | (0.56)              | (0.35)                   | (0.44)                   | (1.31)            | (24.8)            | (15.04)           | (2.87)           | (5.28)            | (1.60)            | 37.5      | 12.5      | 50       |
| 'Duncan' x P. trifoliata] |                      |                          |                          |                   |                   |                   |                   |                   |                   | 37.5      | 12.5      | 50       |
| 'India' lime (2450)       | (0.41)              | (0.89)                   | (0.76)                   | (0.69)            | (21.1)            | (6.01)            | (1.49)           | (4.58)            | (3.17)            | 37.5      | 12.5      | 50       |
| 'Yama-mikan' sour orange (3474) | (1.22)              | (1.25)                   | (1.30)                   | (1.31)            | (23.8)            | (7.37)            | (2.76)           | (5.27)            | (1.88)            | 37.5      | 12.5      | 50       |
| 'Mero' lemon (3892)       | (0.71)              | (0.00)                   | (0.71)                   | (0.00)            | (21.2)            | (1.18)            | (0.84)           | (5.26)            | (0.13)            | 37.5      | 12.5      | 50       |
| 'Australian Finger lime' var. Sanguinea (1484) | (0.74)              | (0.76)                   | (0.61)                   | (0.00)            | (24.2)            | (7.06)            | (3.64)           | (4.46)            | (1.60)            | 37.5      | 12.5      | 50       |
| 'Florida' rough lemon (400) | (0.26)              | (0.26)                   | (0.52)                   | (2.60)            | (9.04)            | (2.00)            | (5.61)           | (3.17)            | (1.82)            | 37.5      | 12.5      | 50       |
| 'Lam's lemon' (3919)      | (1.44)              | (1.61)                   | (1.16)                   | (0.29)            | (7.57)            | (8.93)            | (5.59)           | (2.05)            | (1.82)            | 37.5      | 12.5      | 50       |
| 'Tam's lemon' (2427)      | (0.64)              | (0.87)                   | (1.03)                   | (0.63)            | (17.7)            | (12.50)           | (2.60)           | (4.36)            | (3.63)            | 37.5      | 12.5      | 50       |
| 'Khasi' papeda (3052)     | (0.99)              | (1.02)                   | (1.05)                   | (0.00)            | (2.13)            | (1.07)            | (6.06)           | (0.00)            | (0.00)            | 37.5      | 12.5      | 50       |
| 'Sun Chu Sha' mandarin (4003) | (0.38)              | (0.35)                   | (0.39)                   | (3.5)             | (2.9)             | (4.67)            | (1.16)           | (5.63)            | (2.05)            | 37.5      | 12.5      | 50       |
| 'Kinkoju Unshiu' graft chimera (3816) | (0.39)              | (0.19)                   | (1.11)                   | (0.53)            | (17.6)            | (4.37)            | (1.16)           | (5.63)            | (2.05)            | 37.5      | 12.5      | 50       |
| 'Australian Round lime' (3637) | (0.38)              | (0.35)                   | (0.39)                   | (0.41)            | (5.1)             | (4.96)            | (6.80)           | (28.4)            | (29.8)            | 37.5      | 12.5      | 50       |

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Table 1. (Continued) Growth and disease severity metrics for trees of each of 83 seedling populations from diverse seed-source accessions from the National Clonal Germplasm Repository for Citrus and Dates. Trees were evaluated at the USDA Fort Pierce, FL, farm over 2 weeks late October through Nov. 2015, 6 years after planting in an area with severe huanglongbing and natural challenge with Asian citrus psyllid. Overall health, canopy density, dieback, and leaf-mottle severity were scored using a five-point scale, where five was the worst and one was fully healthy. Other metrics were percent blotchy mottle (% of canopy area), tree canopy volume, and trunk diameter. For each seed-source accession, pedigree estimates are calculated from the four progenitors Citrus species (contributing to development of most citrus scion cultivars) and other genera.

| Common name of seed parent | Overall health (Botanical name of seed parent) | Canopy density (Botanical name of seed parent) | Dieback | Leaf mottle (Botanical name of seed parent) | Leaf mottle (% canopy) | Canopy vol. (Botanical name of seed parent) | Trunk diam (cm) | Trees surviving n/8 (Botanical name of seed parent) | $\times$(sd) | $\times$(sd) | $\times$(sd) | $\times$(sd) | Pedigree |
|----------------------------|----------------------------------|----------------------------------|--------|----------------------------------|-----------------------|----------------------------------|---------------|----------------------------------|----------|----------|----------|----------|---------|
| 'Chinese Box orange' (4107) | (C. maxima (Burm.)) | (0.91) | (0.074) | (0.48) | (1.03) | (9.3) | (242) | (4.26) | (6.57) | (0.04) | 25 25 50 100 |
| 'Som Khovan' mandarin (3752) | (C. grandis (L.) | (0.85) | (0.65) | (1.31) | (1.11) | (38.4) | (0.76) | (57.9) | (0.6) | (1.3) | 27.8 28.1 100 |
| 'Chinese Box orange' (1491) | (C. grandis (L.) | (1.44) | (1.44) | (1.53) | (0.00) | (0.114) | (2.07) | (4.94) | (5.65) | (34.4) | 33.5 100 |
| 'Koji' mandarin (3147) | (C. longispina (L.) | (0.61) | (0.58) | (1.30) | (0.80) | (9.2) | (1.17) | (0.74) | (6.15) | (1.48) | 29.0 29.0 8 |
| 'Tahitian' pummelo x 'Star Ruby' grapefruit (3781) | (C. grandis (L.) | (0.53) | (0.00) | (1.06) | (0.35) | (14.1) | (3.37) | (1.35) | (6.6) | (1.39) | 28.0 29.0 8.8 18.1 |
| 'Robinson' mandarin (3850) | (C. reticulata hybrid | (0.65) | (0.65) | (1.38) | (1.71) | (39.7) | (4.53) | (1.91) | (6.93) | (1.45) | 29.0 29.0 8 |
| 'Hassaku' pummelo hybrid (3942) | (C. reticulata hybrid | (0.80) | (1.00) | (0.16) | (0.89) | (25.7) | (10.89) | (1.91) | (5.81) | (3.68) | 27.9 29.8 50 |
| 'Nacho Daiata' sour orange (2588) | (C. reticulata hybrid | (0.52) | (0.61) | (1.17) | (1.14) | (20.5) | (3.25) | (1.17) | (6.47) | (6.08) | 29.5 32.2 50 50 |
| 'Soh Niantra' mandarin (3260) | (C. reticulata hybrid | (0.29) | (0.50) | (0.96) | (1.00) | (11.5) | (0.23) | (4.29) | (6.0) | (1.75) | 29.5 32.2 50 100 |
| 'Simmon's trifoliata' (3549) | (P. trifoliata) | (0.15) | (0.50) | (1.37) | (0.00) | (0.0) | (1.63) | (0.96) | (2.48) | (5.00) | 29.5 32.2 50 100 |
| 'Kao Pan' pummelo (2242) | (Citrus maxima (Burm.)) | (0.63) | (0.70) | (1.81) | (1.27) | (2.32) | (2.59) | (5.12) | (6.02) | (5.59) | 28.7 31.0 100 |
| 'Talaman' x 'Winged lime' (2320) | (Citrus longispatha (Wester) | (0.63) | (0.71) | (1.58) | (0.48) | (22.5) | (1.99) | (0.66) | (6.69) | (1.95) | 29.5 29.6 50 50 |
| 'Hassaku' pummelo hybrid (3907) | (Citrus hassaku (Poiret) | (0.84) | (1.12) | (1.53) | (1.04) | (29.8) | (4.71) | (1.51) | (6.42) | (6.19) | 29.5 29.6 50 50 |

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Table 1. (Continued) Growth and disease severity metrics for trees of each of 83 seedling populations from diverse seed-source accessions from the National Clonal Germplasm Repository for Citrus and Dates. Trees were evaluated at the USDA Fort Pierce, FL, farm over 2 weeks late October through Nov. 2015, 6 years after planting in an area with severe huanglongbing and natural challenge with Asian citrus psyllid. Overall health, canopy density, dieback, and leaf-mottle severity were scored using a five-point scale, where five was the worst and one was fully healthy. Other metrics were percent blotchy mottle (% of canopy area), tree canopy volume, and trunk diameter. For each seed-source accession, pedigree estimates are indicated from the four progenitor *Citrus* species (contributing to development of most citrus scion cultivars) and other genera.

| Common name of seed parent<sup>v</sup> (CRC accession no.) | Overall health (1–5 scale) | Canopy density (1–5 scale) | Dieback (1–5 scale) | Leaf mottle (% canopy) | Canopy vol. (m<sup>3</sup>) | Trunk diam (cm) | Trees surviving | % blotchy mottle (% canopy) | Tree canopy volume | Trunk diameter | Pedigree<sup>w</sup> | Genus | Species | Common name of seed parent<sup>x</sup> (CRC accession no.) | Overall health (1–5 scale) | Canopy density (1–5 scale) | Dieback (1–5 scale) | Leaf mottle (% canopy) | Canopy vol. (m<sup>3</sup>) | Trunk diam (cm) | Trees surviving | % blotchy mottle (% canopy) | Tree canopy volume | Trunk diameter | Pedigree<sup>v</sup> | Genus | Species | Common name of seed parent<sup>x</sup> (CRC accession no.) | Overall health (1–5 scale) | Canopy density (1–5 scale) | Dieback (1–5 scale) | Leaf mottle (% canopy) | Canopy vol. (m<sup>3</sup>) | Trunk diam (cm) | Trees surviving | % blotchy mottle (% canopy) | Tree canopy volume | Trunk diameter | Pedigree<sup>v</sup> | Genus | Species | Common name of seed parent<sup>x</sup> (CRC accession no.) |
|----------------|------------------|------------------|------------------|------------------|------------------|------------------|----------------|----------------|------------------|------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 'Dweet' tangor (3018) (C. reticulata Blanco) (0.71) (0.48) (1.41) (1.65) (39.8) (1.82) (1.70) | 4.5 | 4.6 | 4.0 | 2.1 | 20 | 1.4 | 3.9 | 4 | 30.0 | 26.0 | 87.5 | 12.5 |
| 'S-281 citrangelo' (3552) (x*Citroneuris sp.) | 4.5 | 4.0 | 2.5 | 3.0 | 25 | 1.3 | 2.0 | 1 | 31.8 | 30.1 | 34 | 16 |
| 'Fremon' mandarin (3558) (C. reticulata Blanco) (0.50) (0.58) (0.29) (1.44) (39.6) (0.67) (0.46) | 4.5 | 4.5 | 1.0 | 1.0 | 0.00 | 0.8 | 2.6 | 1 | 38.5 | 40.0 |
| 'Mountain' citron (3780) (Citrus halimii B.C. Stone) (3.69) (na) | 4.5 | 4.5 | 1.0 | 1.0 | 0.00 | 0.8 | 2.6 | 1 | 38.5 | 40.0 |
| 'Mato Buntan' pummelo (3945) (C. maxima (Burm.) Merr.) (0.71) (1.06) (0.00) (2.12) (45.9) (1.82) (1.70) | 4.5 | 4.3 | 3.0 | 2.5 | 33 | 0.8 | 2.9 | 2 | 29.3 | 28.8 |
| 'Pomelit' pummelo hybrid (4026) (C. maxima (Burm.) Merr.) (0.45) (0.38) (0.92) (5.21) (na) | 4.5 | 4.3 | 3.0 | 2.5 | 33 | 0.8 | 2.9 | 2 | 29.3 | 28.8 |
| 'Egami Buntan' pummelo (3959) (C. maxima (Burm.) Merr.) (0.55) (0.56) (0.58) (0.00) (1.29) (34.9) (1.13) (0.59) | 4.5 | 4.3 | 3.0 | 2.5 | 33 | 0.8 | 2.9 | 2 | 29.3 | 28.8 |
| 'Australian Desert lime' hybrid (4105) (Eremocitrus glauca hybrid (Lindl.) Swingle (0.48) (0.58) (0.58) (0.00) (1.29) (34.9) (1.13) (0.59) | 4.5 | 4.3 | 3.0 | 2.5 | 33 | 0.8 | 2.9 | 2 | 29.3 | 28.8 |
| 'Konejime' sour orange hybrid (3611) (Citrus neo-aurentium Tanaka (0.27) (0.35) (1.68) (1.02) (13.8) (1.16) (0.92) (5.05) (1.23) | 4.5 | 4.3 | 3.0 | 2.5 | 33 | 0.8 | 2.9 | 2 | 29.3 | 28.8 |
| 'Scarlett Emperor' mandarin (3326) (C. reticulata Blanco (0.29) (0.29) (1.41) (36.6) (1.14) (0.58) (6.09) (1.50) | 4.5 | 4.3 | 3.0 | 2.5 | 33 | 0.8 | 2.9 | 2 | 29.3 | 28.8 |
| 'Ex-India' sour orange hybrid (3715) (C. reticulata Blanco (0.35) (0.35) (0.00) (1.78) (14.1) (0.37) (0.92) (5.11) (2.40) | 4.5 | 4.3 | 3.0 | 2.5 | 33 | 0.8 | 2.9 | 2 | 29.3 | 28.8 |
| 'Hamley' × *Chinotto (3149) (C. benikoji hort. ex Tan.) (0.29) (0.29) (1.04) (33.3) (4.27) (1.27) (6.18) (2.11) | 4.5 | 4.3 | 3.0 | 2.5 | 33 | 0.8 | 2.9 | 2 | 29.3 | 28.8 |
| 'Tien Chieh' mandarin (3363) (C. reticulata Blanco) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) (0.00) | 4.5 | 4.3 | 3.0 | 2.5 | 33 | 0.8 | 2.9 | 2 | 29.3 | 28.8 |
| 'Monkey orange' (3564) (C. sinensis (L.) Osbeck) (6.22) (na) | 4.5 | 4.3 | 3.0 | 2.5 | 33 | 0.8 | 2.9 | 2 | 29.3 | 28.8 |
| 'Belady' mandarin (3958) (C. reticulata Blanco) (8.33) (na) | 4.5 | 4.3 | 3.0 | 2.5 | 33 | 0.8 | 2.9 | 2 | 29.3 | 28.8 |
| 'Japanese Prickly ash' (Zanthoxylum ailanthoides Siebold and Zucc.) u | 5.0 | 4.5 | 4.5 | 4.0 | 45 | 0.5 | 2.0 | 1 | 30.8 | 33.6 |
| 'Pineapple' sweet orange (3858) (Citrus sinensis (L.) Osbeck) (6.40) (5.85) | 5.0 | 4.5 | 4.5 | 4.0 | 45 | 0.5 | 2.0 | 1 | 30.8 | 33.6 |
| 'Koster' mandarin (3958) (C. reticulata Blanco) (8.33) (na) | 5.0 | 4.5 | 4.5 | 4.0 | 45 | 0.5 | 2.0 | 1 | 30.8 | 33.6 |
| 'S-281 citrangelo' (3552) (x*Citroneuris sp.) (5.21) (2.69) | 5.0 | 4.5 | 4.5 | 4.0 | 45 | 0.5 | 2.0 | 1 | 30.8 | 33.6 |

*This table is sorted based on overall health, with 1 healthiest score.

<sup>2</sup>Common names, accession no., and taxonomic name of seed-source accessions as specified by the Citrus Variety Collection, Riverside, CA (http://www.citrusvariety.ucr.edu), unless otherwise noted.

<sup>3</sup>Ct values represented as Ct value from qPCR. It is important to realize that Ct and titer are inversely and exponentially related. Data are from Ramadugu et al., 2016.

<sup>4</sup>Pedigree assignments are estimates based on diverse reports and professional judgments.

<sup>5</sup>Seed obtained from the Fruit and Spice Park of Miami/Dade County, FL.

<sup>6</sup>Seeds obtained from Dr. John Ruter of the University of Georgia.
Table 2. Seedling populations from 83 diverse seed-source accessions (from the National Clonal Germplasm Repository for Citrus and Dates) were planted at the USDA Fort Pierce, FL Farm. Trees were evaluated (2 weeks October through Nov. 2015) 6 years after planting with continuous exposure to high populations of Asian citrus psyllid and severe huanglongbing pressure. Data in this table are for progenies of the seed-source accessions considered to have 100% of their pedigree from one of the four progenitor species contributing to most citrus scion cultivars. Where noted, disease severity metrics were scored using a five-point scale, where five is the worst and one is fully healthy.

| Metric                  | Citrus % in pedigree | Mandarin % in pedigree |
|-------------------------|----------------------|------------------------|
|                        | r²  | P value | Slope | r²  | P value | Slope |
| Leaf mottle (1–5)       | 0.112 | 0.006 | +   | 0.020 | 0.251 | +   |
| Leaf mottle (%)         | 0.200 | 2.00E–04 | +   | 0.005 | 0.586 | -   |
| Overall health (1–5)    | 0.296 | 3.00E–05 | +   | 0.169 | 7.00E–04 | -   |
| Canopy density (1–5)    | 0.317 | 1.00E–05 | +   | 0.161 | 9.00E–04 | -   |
| Dieback (1–5)           | 0.143 | 0.002 | +   | 0.219 | 8.00E–05 | -   |
| Canopy volume (m³)      | 0.423 | 5.00E–03 | +   | 0.150 | 0.001 | -   |
| Trunk diameter (cm)     | 0.570 | 2.00E–13 | +   | 0.163 | 8.00E–04 | -   |

*Common names of parental lines with accession no. in parentheses as specified by the Citrus Variety Collection, Riverside, CA (http://www.citrusvariety.ucr.edu).

Table 3. Regressions of growth and health metrics versus pedigree percentage from each progenitor “true” *Citrus* species. Data are for trees of 83 seedling populations from diverse seed-source accessions from the National Clonal Germplasm Repository for Citrus and Dates. Growth measurements and disease severity metrics were evaluated at the USDA Fort Pierce, FL farm, over two weeks late October through Nov. 2015, 6 years after planting in an area with severe huanglongbing and natural challenge with Asian citrus psyllid. Overall health, canopy density, dieback, and leaf-mottle severity were scored using a five-point scale, where five was the worst and one was fully healthy. Other metrics were percent blotchy mottle (% of canopy area), tree canopy volume (m³), and trunk diameter. The slope indicates a positive relationship when increased health or growth is associated with increased pedigree percentage of indicated progenitor species.

| Species: Common name of seed parent* (accession no.) | Overall health (1–5 scale) | Canopy density (1–5 scale) | Dieback (1–5 scale) | Leaf mottle (% of canopy) | Canopy vol. (m³) | Trunk diam. (cm) |
|-----------------------------------------------------|-----------------------------|---------------------------|---------------------|--------------------------|----------------|----------------|
| Mandarin: ‘Tien Chieh’ (CRC 2590), ‘Sunki’ (CRC 3143), ‘Soh Niamtra’ (CRC 3260), ‘Som Keowan’ (CRC 3752), ‘Koster’ (CRC 3958), ‘Sun Chu Sha’ (CRC 4003), ‘Kao Pan’ (CRC 2242), ‘Kao Panne’ (CRC 2248), ‘Reinking’ (CRC 3805), ‘Mato Buntan’ (CRC 3945), ‘Gami Buntan’ (CRC 3959), ‘Diamante’ (CRC 3523), ‘South Coast Field Station’ (CRC 3546), ‘Kalpi’ (CRC 1455), ‘Talamisan’ (CRC 2320), ‘Davao’ (CRC 2427), ‘Khasi’ (CRC 3052) | 4.2* | 4.2 | 3.7 | 3.8 | 41 | 1.7 | 3.5 |
| Citron: ‘Chinese Box Orange’ (CRC 1491 and CRC 4105), ‘Chevalier’s Aeglopsis’ (CRC 2320), ‘Australian Desert lime’ (CRC 2878), ‘Gin Berry’ (CRC 3285), and ‘Chinese Box Orange’ (CRC 1491 and CRC 4107) | 2.5 | 2.2 | 2.0 | 4.3 | 69 | 60.4 | 14.6 |
| Pummelo: ‘Las Vegas’ (CRC 3052), ‘Koster’ (CRC 3958), ‘Sun Chu Sha’ (CRC 4003), ‘Kao Pan’ (CRC 2242), ‘Kao Panne’ (CRC 2248), ‘Reinking’ (CRC 3805), ‘Mato Buntan’ (CRC 3945), ‘Gami Buntan’ (CRC 3959) | 3.7 | 3.6 | 2.8 | 2.7 | 36 | 45.3 | 5.34 |
| ‘Egami Buntan’ (CRC 3959), ‘Japa- | 0.065 | 0.041 | – | 0.008 | 0.476 | -   |
| ‘Sungkul’ (CRC 3052) | 0.83 | 1.64 | 1.39 | 0.75 | 28.8 | 2.00 | (2.62) |
| ‘Sun Chu Sha’ (CRC 4003) | 2.5 | 2.2 | 2.0 | 4.3 | 69 | 60.4 | 14.6 |
| ‘Australian Desert lime’ (CRC 2320), ‘Chevalier’s Aeglopsis’ (CRC 4003), ‘Koster’ (CRC 3958), ‘Sun Chu Sha’ (CRC 4003), ‘Kao Pan’ (CRC 2242), ‘Kao Panne’ (CRC 2248), ‘Reinking’ (CRC 3805), ‘Mato Buntan’ (CRC 3945), ‘Gami Buntan’ (CRC 3959) | 3.7 | 3.6 | 2.8 | 2.7 | 35 | 64.4 | 4.4 |

*Means with standard deviation in parentheses.

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Proc. Fla. State Hort. Soc. 129: 2016.
igned in the pedigree gave weak positively correlated. The regressions for (0.296 and 0.317) and were highly significant (\(P = 3.00E^{-06}\) and 1.00E\(-06\), respectively) and positively correlated. The regressions for overall health and canopy density vs. mandarin in the pedigree gave very weak \(r^2\) values of 0.169 and 0.161, though accompanied by highly significant \(P\) values (7.00E\(-04\) and 9.00E\(-04\), respectively), and the relationship was negative in both cases. There was no statistically significant relationship between overall health and canopy volume for either pummelo or papada.

Regressions for growth metrics (canopy volume and trunk diameter) against percent of individual progenitor species in seed-source accession pedigrees gave the most striking differences. TCV vs. percentage citron in pedigree was highly significant \(\left(\frac{5.00E^{-08}}{3.00E^{-02}}\right)\) with an \(r^2 = 0.423\) and a positive relationship. Regression for trunk diameter vs. percentage citron in seed parent pedigree was also highly significant \(\left(P = 2.00E^{-10}\right)\) with an \(r^2 = 0.570\) and a positive correlation. Greater trunk diameter and canopy volume are generally associated with healthy trees suggesting citron in a tree’s pedigree enhances the ability to modulate and use photosynthesis despite stresses associated with HLB. Across all other metrics vs. pummelo and papada, no significant relationship was observed for either canopy volume or trunk diameter (Table 3).

Interestingly, regression of percent leaf mottle vs. percentage citron in pedigree was also highly significant \(\left(P = 2.00E^{-10}\right)\), \(r^2 = 0.200\) and positively correlated. No significant relationship was observed for progenies of seed-source accessions without a citron background. The ability of citrus trees to display high HLB leaf mottle, but still maintain phloem transport was demonstrated by Fan et al. (2012). In that study, even though both cultivars displayed callose deposition in sieve elements and phloem cell collapse, relatively HLB tolerant ‘Florida rough lemon’ (Citrus jambhiri) maintained better phloem transport than HLB sensitive sweet orange. The ability to maintain good phloem transport with extensive CLas titer is likely critical to good HLB tolerance.

### Conclusions

In summary, the objective of this study was to assess growth and visible disease severity metrics on progenies of 83 seed-source genotypes of Citrus and Citrus relatives to ascertain their relative tolerance to HLB. The healthiest trees with low or absent HLB symptoms were distant relatives across all metrics except for TCV and trunk diameter (where Citrus with citron in the pedigree performed better). Within Citrus, the majority of the healthiest trees (greater trunk diameter, less branch dieback, densest canopies, and largest canopy volume) were those with pedigrees that included C. medica. It is noteworthy that these trees all had substantial titers of CLas (Table 1) and therefore their relative health reflects tolerance rather than resistance to HLB. Trees from Citrus seed-source accessions without citron in their background were generally the least healthy overall with less dense canopies, more branch dieback, smaller trunk diameters, and dramatically smaller TCV. The exceptions were progenies of two C. aurantium accessions that were significantly healthier than all other trees from Citrus without citron in pedigree.

Reports of tolerance to HLB are likely greatly influenced by the growing conditions and specific comparisons to other plant material. Florida rough lemon was widely reported as HLB tolerant (Fan et al., 2012). Though it fared markedly better than the mandarins and sweet orange in this study, ‘Florida rough lemon’ ranked 16th out of 21 citron-derived seed-source accession progenies for overall health. Since rough lemon is highly apomictic the materials are likely the same in the two studies. Though true resistance to HLB is the ultimate goal, tolerance to HLB may be invaluable for short-term survival of the industry both here in the United States and abroad. We propose HLB tolerance means infected trees are able to grow, maintain relatively full canopies, and ultimately in commercial scions produce fruit of acceptable quality and quantity to be profitable. Results from this study may ultimately contribute to: 1) identifying HLB-tolerant molecular markers from citron and incorporating into other market phenotypes through conventional breeding; 2) using biotechnology to implement citron-derived HLB-tolerance; and 3) encouraging breeding of HLB-tolerant acid citrus fruit (lemon or lime-like phenotypes) for good quality and adaptation to regions with high HLB pressure.

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