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Colonization of *Citrus* and *Citrus*-related Germplasm by *Diaphorina citri* (Hemiptera: Psyllidae)

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Abstract. **Huanglongbing (HLB) is a serious and devastating disease of citrus caused by *Candidatus* Liberibacter spp. and vectored by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). The disease has the potential to greatly limit the production of citrus in Florida and other citrus-growing regions worldwide. Current control of *D. citri* and HLB is inadequate, but the identification and incorporation of *D. citri* resistance traits from uncultivated *Citrus* spp. and *Citrus* relatives is seen as a potential disease management strategy. In this study, seedlings of 87 Rutaceae seed-source genotypes, primarily in the orange subfamily Aurantioideae, were assessed in the field for their propensity in a free-choice situation for infestations of natural south Florida populations of *D. citri*. The majority of test populations surveyed hosted all three life stages of *D. citri;* however, there were significant differences among the test populations in the mean ranks for *D. citri* eggs ($F = 3.13$, $df = 86$, $P < 0.0001$), nymphs ($F = 9.01$, $df = 86$, $P < 0.0001$), and adults ($F = 4.21$, $df = 86$, $P < 0.0001$). The only sampled test population that was completely avoided by all life stages of *D. citri* was seedlings of *Casimiroa edulis* Llave et Lex, commonly known as white sapote, which was one of the few plants included in the study belonging to the Rutaceae subfamily Toddalioideae. Although not completely avoided, very low levels of *D. citri* were found on two surveyed test populations of *Poncirus trifoliata* (L.) Raf, seedlings of 'Simmon's trifoliate' and 'Little-Leaf'. *Poncirus trifoliata,* the trifoliate orange, readily forms hybrids with *Citrus* spp., is commonly incorporated into rootstock varieties, and has been used in breeding-advanced scion material. The identification of partial resistance in *P. trifoliata* to *D. citri* could prove useful in future citrus breeding efforts aimed at reducing the incidence and spread of HLB. Determining if there is true resistance to *D. citri* in this and other germplasm sources with a low propensity for infestations in free-choice situations will require no-choice experiments.**

The Asian citrus psyllid, *Diaphorina citri*, is a key pest in most citrus-growing regions around the world. *D. citri* nymphs feed exclusively on young elongating flush and feeding can retard leaf and shoot development (Michaud, 2004; Shivankar et al., 2000). In addition, the production of honey dew by feeding nymphs promotes the growth of sooty mold leading to a reduction in the photosynthetic capacity of the plant (Mead, 1977). However, the devastating economic damage caused by this insect comes from its ability to vector the phloem-limited Gram-negative bacteria, *Candidatus* Liberibacter spp., associated with citrus greening disease or huanglongbing (HLB) (Bové, 2006; Martinez and Wallace, 1967; Mead, 1977). Early in the development of HLB symptoms, leaves of citrus trees exhibit yellowed and/or blotchy mottle patterns followed by the development of chlorosis; trees exhibit stunted growth; and fruit become misshapen and inedible and drop prematurely. In 2 to 3 years trees can degenerate into a non-productive state, leading to the eventual death of the tree in ≈5 to 8 years (Halbert and Manjunath, 2004; Tsai and Liu, 2000). HLB and *D. citri* have been known to occur in Asia for many years (Chen et al., 2010; Lin, 1956) and have subsequently spread to the Indian Ocean islands and the Americas (International Society for Infectious Diseases, 2004, 2005, 2008, 2009, 2010, 2011).

Diaphorina citri was found in Florida in 1998 (Halbert, 1998) and the discovery of HLB followed in 2005 (Halbert, 2005). The disease has seriously impacted Florida citrus fruit production, which between 2007 and 2008 was valued at U.S. $1.76 billion with fresh fruit accounting for U.S. $253 million and processed fruit U.S. $1.50 billion (Rahmani and Hodges, 2009). *D. citri* is continuing to move into other citrus-producing regions in the United States, including California, Arizona, and Texas (da Graça et al., 2008; French et al., 2001), and it is likely that with time the disease will follow the spread of the vector.

The production of disease-resistant varieties is a vital component to a sustainable and long-term citrus management program. Insect-resistant traits have been documented among members of the orange subfamily (Rutaceae: Aurantioideae) (Bowman et al., 2001; Luthria et al., 1989; Yang and Tang, 1988), and it is possible that the Aurantioideae germplasm reservoir contains traits that confer specific resistance to *D. citri*. The Aurantioideae, one of seven subfamilies of the Rutaceae, contains 33 genera and ≈210 species native to the Old World tropics (Engler, 1931). The trees and shrubs of the orange subfamily are distinguished by persistent leaves except in three monotypic genera (*Poncirus*, *Aegle*, and *Feronia*), in several species of *Clausena*, and in one species of *Murraya* (*M. alternans*). Most members of the subfamily produce fruit with a leathery peel or hard shell; have fragrant white flowers; and leaves and fruit contain schizolysigenous oil glands that give off an aroma when touched (Swingle and Reece, 1967). Interspecific and to a lesser extent intergeneric hybridization within *Citrus* and among its relatives is common and has probably taken place in the wild for centuries (Nicolosi, 2007), thus making it difficult to fit the species concept to this group of plants. The taxonomic classification of the members of the Aurantioideae into tribes, subtribes, genera, and species is controversial and unresolved (Barrett and Rhodes, 1976; Bayer et al., 2009; Moore, 2001). Both the Tanaka (Tanaka, 1954, 1977) and Swingle (Swingle 1943; Swingle and Reece, 1967) systems are widely accepted for citrus taxonomy.

Previously published host plant information on *D. citri* suggests that this insect has a broad host range within the Aurantioideae and may even on occasion use Rutaceae species outside the orange subfamily as a food host (Aubert, 1990; Halbert and Manjunath, 2004; Yang et al., 2006). There is also evidence that there are differences in susceptibility to *D. citri* among common *Citrus* cultivars (Nehru et al., 2004; Tsagkarakis and Rogers, 2010; Tsai and Liu, 2000). However, in many of these studies, only a limited number of plants was surveyed often at a single time point, generally only presence or absence was

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recorded, and only limited information was presented on the susceptibility to each *D. citri* life stage. The objective of this study was to survey a Rutaceae planting, primarily composed of Aurantioideae seedling populations derived from diverse species, multiple times during the height of *D. citri* field populations for the presence and abundance of adults, nymphs, and eggs.

Materials and Methods

Seed source. Seeds representing a wide diversity of *Citrus* and *Citrus* relatives in the family Rutaceae were obtained from the National Clonal Germplasm Repository for Citrus and Dates (NCGRCD) at the University of California at Riverside (UCR), Riverside, CA. The seeds were derived from the Citrus Variety Collection of UCR, which was created nearly 100 years ago and contains more than 1000 accessions [each with a unique Citrus Research Center (CRC) number] of cultivated and wild species of *Citrus* and *Citrus* relatives, which are maintained as trees adjacent to the UCR campus. The Core Collection of citrus (Barkley, 2003) was included as seed sources also, because this subcollection of genotypes in the Citrus Variety Collection represents ≈85% of the genetic diversity as determined using 23 simple sequence repeat markers. Details of CRC genotypes used in this study can be obtained from the Citrus Variety Collection web site at http://www.citrusvariety.ucr.edu. All accessions from NCGRCD were members of the orange subfamily Aurantioideae, Rutaceae. Two more distant members of the subfamily Aurantioideae, *Afraegle paniculata* (Schum.) Engl. and *Aegle marmelos* (L.) Corr., as well as *Casimiroa edulis* (Llave et Lex), which is in the subfamily Toddalioideae, Rutaceae, were obtained from the Fruit and Spice Park of Miami/Dade County. Seeds of an additional Toddalioideae species, *Zanthoxylum ailanthoides* (L.), were obtained indirectly from Dr. John Ruter of the University of Georgia.

Seeds of 124 CRC accessions as well as additional material described previously were received and planted in a greenhouse at the USDA-ARS Laboratory in Fort Pierce, FL, in individual plastic cells (3.8 × 21 cm) (SC-10 super cell Cone-tainers; Stuewe and Sons, Corvallis, OR) containing sterile soil. Seedlings from 87 seed-source genotypes (hereafter referred to as test populations in the text) were successfully propagated. It must be noted that members of the Rutaceae vary greatly in their incidence of nucellar embryony (reviewed in Frost and Soost, 1968) and so some of the seedling test populations were essentially genetically identical to the seed parent, whereas others represented half-sib families with only the seed-parent known. Seedlings were transplanted to 3.7-L containers when they were 4 to 7 months old and maintained on elevated benches in a greenhouse with an average diurnal temperature cycle of 35 °C maximum and 23 °C minimum in the summer and a diurnal cycle of 32 °C and 20 °C in the winter. No supplemental light was supplied. Plants were watered with a dilute fertilizer mix weekly using water-soluble N:P:K (20:10:20) at a rate of 150 mg·L^{-1} N.

Field evaluation for D. citri *colonization.* After 6 to 9 months in the greenhouse, eight single-plant replicates for each seedling test population were planted in a randomized complete block design in a plot with trees spaced at 0.6 m between trees in the row and 3.5 m between rows on a USDA-ARS research farm located near Fort Pierce, FL, in Saint Lucie County with most plants germinated in Aug. 2008 and field-planted during June and July 2009. The planting was regularly irrigated and no pesticides were applied to the plants during the study. Plants were fertilized using a program similar to that used for a new commercial planting of citrus. Plants were surveyed four times for *D. citri* infestations, approximately once per month from June to September of 2010.

Because the presence of immature *D. citri* is dependent on the presence of suitable flush, plants were sampled only if a minimum of one flush shoot was present. If no flush was present, then the plant replicate was excluded from the survey during that sampling period. Flush was defined as any shoot with developing leaves, which included breaking buds of new, unexpanded flush to shoots with expanded but tender young leaves (Hall and Albrigo, 2007). Overall plant and flush morphology of the test populations differed and a careful examination of each plant to locate flush was often necessary. Eggs, nymphs, and adult abundances were recorded as categorical counts based on a 0 to 3 ordinal scale. Egg sample categories per flush shoot were 0 = no eggs present; 1 = from one to 20 eggs; 2 = from 21 to 40 eggs; and 3 = more than 40 eggs. Nymph sample categories per flush shoot were 0 = no nymphs; 1 = from one to 10 nymphs; 2 = from 11 to 30 nymphs; and 3 = more than 30 nymphs. Adult sample categories per plant were 0 = no adults present; 1 = from one to five; 2 = from six to 15; and 3 = more than 15 adults. Egg, nymph, and adult density assignments to each categorical count were based on empirical observations of infestation densities in conjunction with published information (Hall et al., 2008).

For plants with multiple flush shoots, the first shoot examined that contained the life stage under assessment was used to determine the estimate per flush shoot for that replicate. For example, if the first flush shoot examined contained eggs, then the egg count for that shoot was the eggs per shoot estimate for that replicate. If, however, the shoot contained no eggs, subsequent flush shoots were examined until eggs were found or until all shoots were sequentially and exhaustively examined and it was determined no eggs were present on the plant. This was the same method used to count nymphs and additionally, if both eggs and nymphs were found on the same flush shoot, then both life stages were counted from the one shoot. Shoots were not removed and counts were made with as little disturbance of plant tissue and insects as possible while maintaining accuracy. The assessment of adult abundance was based on a 30-s examination of the entire plant, including flush, after which an entire plant adult count was recorded.

Statistical analyses. The counts from the 87 test populations collected over the 4 sampling months were analyzed as non-parametric repeated measure data using the F-approximation of the Friedman test (Ipe, 1987) and the associated rank sum multiple comparison test with PROC GLM in SAS (SAS Institute, 2008). The procedure was used after ranking the data within each sampling date from lowest to highest value using the PROC RANK procedure in SAS. The analyses of count data associated with each life stage (egg, nymph, and adult) were carried out separately.

Results

There were significant differences in abundance of *D. citri* eggs ($F = 3.13$, $df = 86$, $P < 0.0001$), nymphs ($F = 9.01$, $df = 86$, $P < 0.0001$), and adults ($F = 4.21$, $df = 86$, $P < 0.0001$) among the 87 test populations according to the Friedman test. Mean ranks, group mean separations resulting from pairwise comparisons, and mean counts (zero to three) for the three life stages on test populations from the 87 seed sources are listed in Table 1. The overall order of seed-source genotypes in Table 1 is based on descending infestations of test populations by adult *D. citri*. All eight replicates did not always produce flush during each of the four sampling periods, so the total number of plants surveyed (designated by "N" in the column in Table 1) for each test population was often not the maximum 32 possible over the entire study period. *Clausena excavata* (CRC 3166), *Balsamocitrus dawei* (CRC 3514), *Glycosmis pentaphylla* (CRC 3285), and *Casimiroa edulis* were the only seed sources providing test populations in which all eight replicates could be surveyed four times.

Of the two test populations surveyed in the subfamily Toddalioideae, seedlings produced from *C. edulis* were completely avoided by *D. citri*, whereas *Zanthoxylum ailanthoides* seedlings were only host to adult *D. citri*. Many test populations from seed-source genotypes showed variability in mean rank among the *D. citri* life stages. Thus, a test population from a seed source with a high mean rank for eggs may have a lower mean rank for nymphs or adults. Table 2 lists the seed-source genotypes with test populations least colonized for each of the *D. citri* life stages: mean separation statistical group "P" for adults, group "R" for nymphs, and group "H" for eggs (refer to Table 1 for statistical groupings). A majority of the test populations that were least colonized were not in the *Citrus* genus. Six seed sources providing test populations fell into the lowest susceptibility group for all three life stages: *C. edulis*, *Poncirus trifoliata* (CRC 4007), *P. trifoliata* (CRC 3549), *Glycosmis pentaphylla* (CRC 3285), *Clausena harmandiana* (CRC 4034), and *Z. ailanthoides*.

Table 3 lists the seed-source genotypes providing test populations most heavily colonized by *D. citri*, which from Table 1 fell in

Table 1. Colonization of field-planted seedling test populations of *Citrus* and *Citrus* relatives to *Diaphorina citri* surveyed in Ft. Pierce, FL.[z]

Botanical name of seed parent[y] (CRC[x])	Common name of seed parent	N	Mean adult rank[w]	Mean count adults (0–3)[v]	Mean nymph rank[w]	Mean count nymphs (0–3)[v]	Mean egg rank[w]	Mean count eggs (0–3)[v]
[u]*Citrus reticulata* Blanco (2590)	Tien Chieh mandarin	27	446.4^{A}	1.11	350.6^{BCDEFG}	1.63	352.1^{ABC}	1.11
Bergera koenigii L. (3165)	Curry tree	31	427.3^{AB}	1.35	386.9^{ABCDEF}	1.81	358.5^{ABC}	1.29
Murraya paniculata (L.) Jack (1637)	Orange Jessamine	29	399.9^{ABC}	1.07	333.0^{CDEFGHI}	1.48	368.6^{ABC}	1.17
[u]*C. maxima* (Burm.) Merr. (3945)	Mato Buntan pummelo	21	386.8^{ABCD}	0.90	297.9^{GHIJKL}	1.38	343.0^{ABC}	1.19
C. medica L. (3523)	Diamante citron	31	376.7^{ABCD}	0.97	384.5^{ABCDEFG}	1.81	304.5^{CDE}	1.00
[u]*C. macrophylla* Wester (3842)	Alemow	29	375.8^{ABCD}	0.83	373.1^{ABCDEFG}	1.72	386.5^{AB}	1.48
[u]*C. limonia* (L.) Osbeck (712)	Santa Barbara red lime	31	368.9^{BCD}	0.81	398.7^{ABCD}	1.94	365.9^{ABC}	1.26
[u]*C. limettioides* Tan. (1482)	Palestine sweet lime	24	365.6^{BCD}	0.75	400.4^{ABCD}	1.88	298.4^{CDE}	0.79
[u]*C. reticulata* Blanco (3752)	Som Keowan mandarin	28	365.6^{BCD}	0.75	357.4^{ABCDEFG}	1.57	346.7^{ABC}	1.00
[u]*C. aurantiifolia* (Christm.) Swing. (2450)	India lime	29	362.3^{BCD}	0.83	379.0^{ABCDEFG}	1.79	303.0^{CDE}	0.86
C. reticulata Blanco (3260)	Soh Niamtra mandarin	31	361.6^{BCD}	0.87	364.9^{ABCDEFG}	1.65	319.3^{ABCD}	0.90
[u]*C. limon* (L.) Burm.*f.* (3176)	Frost nucellar Lisbon	26	357.4^{BCD}	0.81	370.9^{ABCDEFG}	1.73	345.2^{ABC}	1.08
[u]*C. aurantium* L. (2717)	Olivelands sour orange	25	357.3^{BCD}	0.76	366.4^{ABCDEFG}	1.72	296.5^{CDE}	0.80
C. hybrid (53-1-16 'Clem' × 'Hamlin') × *Chinotto* F1 (3715)	Sour orange hybrid ex-India	25	357.1^{BCD}	0.76	320.7^{DEFGHIJK}	1.36	336.7^{ABCD}	1.08
[u]*C. reticulata* Blanco (3558)	Fremont mandarin	25	355.0^{BCDE}	0.76	322.7^{CDEFGHIJK}	1.40	271.6^{CDEF}	0.68
[u]*C. medica* L. (3546)	South Coast Field Station citron	29	353.8^{BCDE}	0.86	425.7^{AB}	2.07	317.5^{ABCD}	1.00
[u]*C. limon* (L.) Burm.*f.* (3885)	Local variety from Iran	27	353.8^{BCDE}	0.85	387.0^{ABCDEF}	1.81	333.0^{ABCD}	1.04
C. sunki hort. ex Tan. (3143)	Sunki mandarin	30	352.5^{CDE}	0.80	316.5^{EFGHIJK}	1.37	310.9^{BCDE}	0.87
C. davaoensis (Wester) Tan. (2427)	Davao lemon (Papeda)	27	346.5^{CDEF}	0.78	361.0^{ABCDEFG}	1.70	315.9^{ABCD}	0.93
[u]*C. hassaku*, hort ex Tan. (3942)	Hassaku pummelo hybrid	30	344.1^{CDEF}	0.73	381.7^{ABCDEFG}	1.83	338.2^{ABC}	1.10
C. volkameriana/C. limonia Osbeck (3050)	Volkamer lemon hybrid	26	343.7^{CDEFG}	0.73	369.9^{ABCDEFG}	1.69	294.5^{CDE}	0.73
[u]*C. limon* L. Burm.*f.* (3005)	Frost nucellar Eureka lemon	26	343.0^{CDEFG}	0.77	314.0^{EFGHIJK}	1.38	317.3^{ABCD}	1.08
[u]*C. reticulata* Blanco (3022)	Frua mandarin	26	340.1^{CDEFGH}	0.73	301.1^{GHIJK}	1.27	302.1^{CDE}	0.81
×*Citrofortunella* sp. (3172)	Tavares limequat	29	339.9^{CDEFGH}	0.72	301.6^{GHIJK}	1.28	318.4^{ABCD}	0.86
C. aurantium L. (3929)	Gou Tou Cheng	30	339.1^{CDEFGH}	0.70	309.0^{GHIJK}	1.30	336.4^{ABCD}	1.03
[u]*C. medica* L. (661)	Indian citron hybrid	26	333.9^{CDEFGHI}	0.73	337.3^{CDEFGH}	1.54	246.5^{DEFGH}	0.46
[u]*C. neo-aurantium* (*C. obovoidea* + *C. unshiu* graft chimera) (3816)	Kinkoji Unshiu graft chimera	28	330.1^{CDEFGHI}	0.68	377.2^{ABCDEFG}	1.75	352.2^{ABC}	1.18
[u]*C. reticulata* Blanco (3958)	Koster mandarin	29	321.8^{DEFGHI}	0.69	361.1^{ABCDEFG}	1.72	331.0^{ABCD}	1.03
×*Citroncirus* sp. (3552)	S-281 Citrangelo	29	321.5^{DEFGHI}	0.66	346.4^{CDEFG}	1.55	314.9^{ABCD}	0.83
[u]*C.maxima* (Burm.) Merr. (2248)	Kao Panne pummelo	28	319.5^{DEFGHI}	0.61	359.5^{ABCDEFG}	1.75	318.0^{ABCD}	1.00
[u]*C.excelsa* Wester (2317)	Limon Real	26	317.3^{DEFGHIJ}	0.62	346.9^{BCDEFG}	1.58	322.2^{ABCD}	0.96
C. paradisi Macf. (3781)	Tahitian pummelo × Star Ruby grapefruit	27	316.9^{DEFGHIJ}	0.63	385.4^{ABCDEFG}	1.89	290.8^{CDE}	0.81
Murraya paniculata L. (3171)	var. ovatifoliolata	30	316.5^{DEFGHIJ}	0.70	294.4^{GHIJKL}	1.20	345.8^{ABC}	1.03
C. amblycarpa Och. (2485)	Nasnaran mandarin	29	313.9^{DEFGHIJ}	0.62	302.1^{GHIJK}	1.28	336.7^{ABCD}	1.03
C. reticulata Blanco (300)	Parson's Special mandarin	28	311.4^{DEFGHIJK}	0.54	248.8^{IJKLMNO}	0.93	288.0^{CDE}	0.71

(*Continued on next page*)

Table 1. (*Continued*) Colonization of field-planted seedling test populations of *Citrus* and *Citrus* relatives to *Diaphorina citri* surveyed in Ft. Pierce, FL.[z]

Botanical name of seed parent[y] (CRC[x])	Common name of seed parent	N	Mean adult rank[w]	Mean count adults (0–3)[v]	Mean nymph rank[w]	Mean count nymphs (0–3)[v]	Mean egg rank[w]	Mean count eggs (0–3)[v]
[u]*C. hassaku* hort ex Tan. (3907)	Hassaku pummelo hybrid	26	310.4^{DEFGHIJK}	0.54	374.9^{ABCDEFG}	1.77	289.9^{CDE}	0.77
C. reticulata (3326)	Scarlet Emperor mandarin	25	309.0^{DEFGHIJK}	0.56	249.8^{JKLMNO}	0.96	329.7^{ABCD}	1.08
C. reticulata Blanco (4003)	Sun Chu Sha mandarin	29	308.2^{DEFGHIJK}	0.55	352.7^{ABCDEFG}	1.62	341.5^{ABC}	1.03
C. intermedia hort ex Tan. (3474)	Yama-mikan sour orange	27	307.7^{DEFGHIJK}	0.52	286.7^{GHIJKL}	1.15	314.5^{ABCD}	0.93
[u]*C. maxima* (Burm.) Merr. (2242)	Kao Pan pummelo	27	307.1^{DEFGHIJK}	0.56	361.7^{ABCDEFG}	1.67	326.8^{ABCD}	1.15
[u]*C. limon* (L.) Burm.*f* (3593)	Interdonato lemon	29	306.8^{DEFGHIJK}	0.59	365.1^{ABCDEFG}	1.66	309.3^{BCDE}	0.93
[u]*C.nobilis* Lour. (3845)	King tangor	27	302.3^{DEFGHIJKL}	0.52	273.3^{GHIJKLM}	1.11	261.5^{CDEFG}	0.59
C. limonia (L.) Osbeck (3919)	Lamas rangpur lime	28	301.5^{DEFGHIJKL}	0.61	393.1^{ABCDE}	1.89	334.1^{ABCD}	1.11
×*Citroncirus* sp. (*C. paradisi* 'Duncan' × *P. trifoliata*) (3771)	Swingle citrumelo	32	300.1^{DEFGHIJKL}	0.56	427.2^{A}	2.09	277.2^{CDE}	0.63
[u]*C. maxima* (Burm.) Merr. (4026)	Pomelit Pummelo hybrid	29	299.9^{DEFGHIJKL}	0.55	361.5^{ABCDEFG}	1.72	313.2^{ABCD}	0.97
[u]*C. limon* (L.) Burm.*f.* (3892)	Mesero lemon	27	298.1^{DEFGHIJKLM}	0.63	316.9^{EFGHIJK}	1.41	327.1^{ABCD}	0.93
[u]*C. aurantiifolia* (Christm.) Swing. (3822)	Mexican lime type	28	297.1^{DEFGHIJKLM}	0.57	402.2^{ABC}	1.93	311.3^{BCDE}	0.89
C. reticulata Blanco (3363)	Belady mandarin	31	296.7^{DEFGHIJKLM}	0.52	298.5^{GHIJK}	1.23	286.0^{CDE}	0.71
C. maxima (Burm.) Merr. (3805)	Reinking	28	295.6^{DEFGHIJKLMN}	0.57	310.4^{FGHIJK}	1.36	338.3^{ABC}	1.07
C. reticulata Blanco (3812)	Unnamed mandarin	27	294.9^{DEFGHIJKLMN}	0.52	259.5^{HIJKLMNO}	1.00	278.0^{CDE}	0.67
C. benikoji hort. ex Tan. (3149)	*C. benikoji*	26	294.3^{DEFGHIJKLMN}	0.54	255.0^{IJKLMNO}	1.04	299.5^{CDE}	0.77
Afraegle paniculata (Schum.) Engl. (–)	Nigerian powder flask fruit	31	293.9^{DEFGHIJKLMN}	0.55	297.0^{GHIJKL}	1.26	391.3^{A}	1.45
C. aurantium L. (3930)	Zhuluan sour orange hybrid	23	293.8^{DEFGHIJKLMN}	0.48	330.3^{CDEFGHIJ}	1.48	328.3^{ABCD}	0.96
[u]*C. reticulata* hybrid (Clementine × Orlando) (3850)	Robinson mandarin	29	290.6^{DEFGHIJKLMN}	0.55	254.5^{JKLMNO}	0.90	273.2^{CDE}	0.62
C. jambhiri Lush. (400)	Florida rough lemon	30	290.1^{DEFGHIJKLMN}	0.53	309.9^{FGHIJK}	1.37	272.7^{CDEF}	0.70
C. taiwanica Tan. & Shimada (2588)	Nansho Daidai sour orange	29	286.1^{DEFGHIJKLMN}	0.48	425.0^{AB}	2.07	392.4^{A}	1.34
[u]*C. reticulata* (Clementine × Orlando) (3851)	Lee mandarin	23	283.3^{DEFGHIJKLMN}	0.43	277.4^{GHIJKLM}	1.04	281.9^{CDE}	0.74
Clausena excavata Burm. *f.* (3166)	Pink wampee	32	278.8^{DEFGHIJKLMN}	0.56	241.9^{KLMNO}	0.94	259.3^{CDEFG}	0.66
C. lycopersiciformis hort ex Tan. (3564)	Monkey orange	28	278.8^{DEFGHIJKLMN}	0.46	247.7^{KLMNO}	1.00	338.3^{ABC}	1.04
[u]*C. aurantium* L. (3289)	Sour orange var. salicifolia	7	278.7^{DEFGHIJKLMNO}	0.43	261.6^{GHIJKLMNO}	1.00	249.7^{CDEFGH}	0.43
C. latipes (Swing.) Tan. (3052)	Khasi papeda	26	277.3^{EFGHIJKLMNO}	0.42	257.9^{HIJKLMNO}	1.04	288.6^{CDE}	0.81
[u]*C. maxima* (Burm.) Merr. (3959)	Egami Buntan pummelo	30	277.1^{FGHIJKLMNO}	0.50	344.8^{CDEFG}	1.60	352.3^{ABC}	1.20
C. webberi Wester (1455)	Kalpi papeda	29	276.5^{FGHIJKLMNO}	0.45	358.1^{ABCDEFG}	1.66	309.4^{BCDE}	0.93
C. reticulata Blanco (3018)	Dweet tangor	30	275.8^{FGHIJKLMNO}	0.47	293.8^{GHIJKL}	1.27	266.6^{CDEF}	0.67
[u]*C. neo-aurantium* Tan. (3611)	Konejime sour orange hybrid	26	274.2^{FGHIJKLMNO}	0.42	345.1^{CDEFG}	1.54	303.0^{CDE}	0.85
Severinia buxifolia (Poiret) Tan. (1497)	Chinese box orange (brachytic form)	25	270.5^{FGHIJKLMNO}	0.52	159.3^{OPQR}	0.48	256.8^{CDEFG}	0.64
Microcitrus australis (Planch.) Swing. (3673)	Australian round lime	26	267.8^{GHIJKLMNO}	0.38	237.6^{KLMNO}	0.92	276.0^{CDE}	0.58

(*Continued on next page*)

Table 1. (*Continued*) Colonization of field-planted seedling test populations of *Citrus* and *Citrus* relatives to *Diaphorina citri* surveyed in Ft. Pierce, FL.[z]

Botanical name of seed parent[y] (CRC[x])	Common name of seed parent	N	Mean adult rank[w]	Mean count adults (0–3)[v]	Mean nymph rank[w]	Mean count nymphs (0–3)[v]	Mean egg rank[w]	Mean count eggs (0–3)[v]
C. longispina Wester (2320)	Talamisan	27	$264.6^{HIJKLMNO}$	0.37	$264.0^{GHIJKLMN}$	1.04	332.4^{ABCD}	0.96
×*Citroncirus* sp.(*Cleopatra mandarin* × *trifoliate*) (3957)	X639 trifoliate hybrid	31	$260.0^{IJKLMNO}$	0.39	219.3^{LMNO}	0.77	328.3^{ABCD}	0.97
C.sinensis (L.) Osbeck (3858)	Pineapple sweet orange	28	$259.6^{IJKLMNO}$	0.39	280.1^{GHIJKL}	1.21	256.5^{CDEFG}	0.57
×*Citroncirus* sp. (301)	Rusk citrange trifoliate hybrid	30	$259.1^{IJKLMNO}$	0.43	301.6^{GHIJK}	1.27	271.2^{CDEF}	0.60
Balsamocitrus dawei Stapf (3514)	Uganda Powder -flask	32	245.3^{JKLMNO}	0.35	189.7^{NOP}	0.62	277.1^{CDE}	0.79
×*Microcitronella* sp. (*M. australasica* × Calamondin) (1466)	Faustrimedin	26	239.1^{JKLMNO}	0.27	195.8^{MNO}	0.65	311.3^{ABCDE}	0.96
Aegle marmelos (L.) Corr. (3140)	Indian Bael fruit	26	234.6^{KLMNO}	0.27	175.6^{OPQ}	0.50	228.8^{FGH}	0.46
C. aurantium L. (628)	Standard sour orange	28	226.7^{LMNOP}	0.29	$261.0^{HIJKLMNO}$	1.14	291.8^{CDE}	0.86
C. leiocarpa hort ex Tan. (3147)	Koji mandarin	24	226.0^{LMNOP}	0.25	198.2^{MNO}	0.71	257.4^{CDEFG}	0.75
Microcitrus hybrid (*M. australis* × *M. australasica*) (1485)	Sydney Hybrid	31	224.6^{MNOP}	0.26	352.4^{BCDEFG}	1.68	368.3^{ABC}	1.26
Zanthoxylum ailanthoides L. (–)	Japanese prickly -ash	31	216.5^{MNOP}	0.29	91.3^{R}	0.00	153.2^{H}	0.00
Microcitrus australasica (F.J. Muell.) Swing. (1484)	Australian finger lime var. Sanguinea	21	216.2^{MNOP}	0.24	173.5^{OPQR}	0.48	254.0^{CDEFG}	0.62
[u]*C. halimii* B.C. Stone (3780)	*Citrus halimii*	22	214.3^{NOP}	0.27	190.8^{MNOP}	0.64	272.7^{CDEF}	0.73
Eremocitrus glauca (Lindley) Swing. Hybrid (4105)	Australian desert lime hybrid	30	208.4^{NOP}	0.20	232.7^{KLMNO}	0.90	236.4^{DEFGH}	0.50
Clausena harmandiana (Pierre) Guillaumin (4034)	*Clausena harmandiana*	26	206.4^{NOP}	0.19	111.2^{PQR}	0.15	181.3^{GH}	0.23
Microcitrus inodora (F.M. Bail) Swing. (3785)	Large leaf Australian wild lime	14	203.2^{NOP}	0.14	225.9^{KLMNO}	0.86	236.3^{DEFGH}	0.64
Glycosmis pentaphylla (Retz.) Corr. (3285)	Orangeberry/Gin berry	32	203.0^{OP}	0.16	96.9^{R}	0.03	162.2^{H}	0.03
[u]*Poncirus trifoliata* L. (3549)	Simmons trifoliate	28	179.8^{OP}	0.07	95.7^{R}	0.04	160.3^{H}	0.04
Poncirus trifoliata L. (4007)	"Little-Leaf" trifoliate	31	175.4^{OP}	0.06	100.4^{QR}	0.03	189.1^{FGH}	0.16
Casimiroa edulis Llave et Lex (–)	White Sapote	32	158.9^{P}	0.00	92.0^{R}	0.00	154.1^{H}	0.00

[z]Data are isted in order of decreasing colonization by adults. Members of the Rutaceae vary greatly in their incidence of nucellar embryony (reviewed in Frost and Soost, 1968) and so some of the plants tested were essentially genetically identical to the seed parent, whereas others represent half-sib families with only the seed parent known.

[y]Botanical and common names of source material as specified by UC Riverside Citrus Variety Collection, Riverside, CA, as defining specific accession number more detailed information can be found at http://www.citrusvariety.ucr.edu. parent.

[x]UC Riverside Citrus Variety Collection, Riverside, CA, accession number.

[w]Mean rank was calculated using the nonparametric Friedman's test with PROC GLM procedure in SAS and letters denoted shared groups based on pairwise comparisons of ranks.

[v]Details of the 0–3 rating scale for adults, nymphs and eggs is provided in the text.

[u]Botanical names from the Core Collection of Citrus, representing a broad range of genetic diversity as determined by simple sequence repeat markers (Barkley, 2003).

the mean separation statistical "A" group for each life stage. Two test populations fell into the "A" group for each *D. citri* life stage: seedlings of *Bergera koenigii* (CRC 3165) and *Citrus macrophylla* (CRC 3842). The majority of the test populations exhibiting high susceptibility to *D. citri* were from seed sources in the *Citrus* genus, but the following non-*Citrus* test populations were also heavily colonized: seedlings of *B. koenigii*, *Murraya paniculata* (CRC 1637), ×*Citroncirus* sp. (CRC 3771), *Afraegle paniculata*, *Microcitrus* hybrid (CRC 1485), *M. paniculata* (CRC 3171), ×*Citroncirus* sp. (CRC 3957), ×*Citrofortunella* sp. (CRC 3172), ×*Citroncirus* sp. (CRC 3552), and ×*Microcitronella* sp. (CRC 1466).

Discussion

In this study, several test populations were identified that were avoided as a host plant by *D. citri*. Of the six test populations that were least colonized by all three life stages, only seedlings from the genus *Poncirus* Raf. were in the "true citrus fruit trees," a group designated by Swingle and thus sexually compatible with *Citrus*. Swingle considered the monotypic genus *Poncirus* to be the most genetically isolated and aberrant of the "true citrus fruit trees" as a result of characters such as trifoliate deciduous leaves and cold-hardiness (Swingle, 1943; Swingle and Reece, 1967). However, recent phylogenetic work on the genera of the

Table 2. Test populations colonized least by each life stage of *D. citri* among seedlings of 87 seed-source genotypes of *Citrus* and *Citrus* relatives surveyed in Ft. Pierce, FL.[z]

Botanical name of seed parent	Common name of seed parent	CRC	Mean rank	Mean count (0–3)
Adults				
Casimiroa edulis Llave et Lex	White Sapote	—	158.9	0.00
Poncirus trifoliata L.	"Little-Leaf" trifoliate	4007	175.4	0.06
Poncirus trifoliata L.	Simmons trifoliate	3549	179.8	0.07
Glycosmis pentaphylla (Retz.) Corr.	Orangeberry/Gin berry	3285	203.0	0.16
Microcitrus inodora (F.M. Bail) Swing.	Large leaf Australian wild lime	3785	203.2	0.14
Clausena harmandiana (Pierre) Guillaumin	*Clausena harmandiana*	4034	206.4	0.19
Eremocitrus glauca (Lindley) Swing. hybrid	Australian desert lime hybrid	4105	208.4	0.20
Citrus halimii B.C. Stone	*Citrus halimii*	3780	214.3	0.27
Microcitrus australasica (F.J. Muell.) Swing.	Australian finger lime var. Sanguinea	1484	216.2	0.24
Zanthoxylum ailanthoides L.	Japanese prickly-ash	—	216.5	0.29
Microcitrus hybrid (*M. australis* × *M. australasica*)	Sydney Hybrid	1485	224.6	0.26
Citrus leiocarpa hort ex Tan.	Koji mandarin	3147	226.0	0.25
Citrus aurantium L.	Standard sour orange	628	226.7	0.29
Nymphs				
Zanthoxylum ailanthoides L.	Japanese prickly-ash	—	91.3	0.00
Casimiroa edulis Llave et Lex	White Sapote	—	92.0	0.00
Poncirus trifoliata L.	Simmons trifoliate	3549	95.7	0.04
Glycosmis pentaphylla (Retz.) Corr.	Orangeberry/Gin berry	3285	96.9	0.03
Poncirus trifoliata L.	"Little-Leaf" trifoliate	4007	100.4	0.03
Clausena harmandiana (Pierre) Guillaumin	*Clausena harmandiana*	4034	111.2	0.15
Severinia buxifolia (Poiret) Tan.	Chinese box orange (brachytic form)	1497	159.3	0.48
Microcitrus australasica (F.J. Muell.) Swing.	Australian finger lime var. Sanguinea	1484	173.5	0.48
Eggs				
Zanthoxylum ailanthoides L.	Japanese prickly-ash	—	153.2	0.00
Casimiroa edulis Llave et Lex	White Sapote	—	154.1	0.00
Poncirus trifoliata L.	Simmons trifoliate	3549	160.3	0.04
Glycosmis pentaphylla (Retz.) Corr.	Orangeberry/Gin berry	3285	162.2	0.03
Clausena harmandiana (Pierre) Guillaumin	*Clausena harmandiana*	4034	181.3	0.23
Poncirus trifoliata L.	"Little-Leaf" trifoliate	4007	189.1	0.16
Aegle marmelos (L.) Corr.	Indian Bael fruit	3140	228.8	0.46
Microcitrus inodora (F.M. Bail) Swing.	Large leaf Australian wild lime	3785	236.3	0.64
Eremocitrus glauca (Lindley) Swing. hybrid	Australian desert lime hybrid	4105	236.4	0.50
Citrus medica L.	Indian citron hybrid	661	246.5	0.46
Citrus aurantium L.	Sour orange var. salicifolia	3289	249.7	0.43

[z]For each life stage, seed-source genotypes are listed in order of increasing colonization of their seedling test populations. Members of the Rutaceae vary greatly in their incidence of nucellar embryony (reviewed in Frost and Soost, 1968) and so some of the plants tested were essentially genetically identical to the seed parent, whereas others represent half-sib families with only the seed parent known.

Aurantioidea keeps *P. trifoliata* in an isolated position but nested within a newly constructed monophyletic *Citrus* clade, renaming it *Citrus trifoliata* (Bayer et al., 2009). Consensus dendrograms further indicate that *P. trifoliata* may be more closely related to certain cultivated species of *Citrus* than other genera of the "true citrus fruit trees" such as *Microcitrus*, *Eremocitrus*, and *Clymenia* (Bayer et al., 2009). Further exploration of the basis of avoidance of *P. trifoliata* by *D. citri* observed in this study would be a worthwhile investment because of the important role of *P. trifoliata* in citrus breeding programs. *Poncirus trifoliata* is graft-compatible and hybridizes with *Citrus*, is the dominant rootstock in China, and since 1892 in Florida has been used as a rootstock alone or in hybrid form (Krueger and Navarro, 2007; Ziegler and Wolfe, 1981). It is an important parent in intergeneric hybrids with *Citrus*, which are collectively referred to as ×*Citroncirus* (Krueger and Navarro, 2007). A number of advanced selections with *Poncirus trifoliata* in their pedigree are present in citrus breeding programs; use of *P. trifoliata* has been largely directed at cold-hardiness, but it is possible that some of these selections, which approach commercial fruit quality, may have *D. citri* resistance as well.

Four ×*Citroncirus* sp. were included among the 87 seed sources providing test populations listed in Table 1: S-281 citrangelo (CRC 3552), Swingle citrumelo (CRC 3771), X639 (CRC 3957), and Rusk (CRC 301). Three of these seed-source genotypes—Swingle citrumelo, X641, and S-281 citrangelo—are also listed in Table 3 as a result of the high affinity of their test populations for *D. citri*. Based on our findings, seedlings of the ×*Citroncirus* seed-source genotypes did not seem to inherit the apparent resistance found in pure *P. trifoliata*, but this may not be true for all hybrids. A further examination of a broader variety of *P. trifoliata* selections and ×*Citroncirus* is currently underway.

Seedling test populations from species within other genera among the "true citrus fruit trees" that were surveyed included *Eremocitrus glauca* (CRC 4105), *Microcitrus australasica* (CRC 1484), *M. australis* (3673), *M.* hybrid (CRC 1485), *M. inodora* (CRC 3785), *Severinia buxifolia* (CRC 1497), ×*Citrofortunella* sp. (CRC 3172), and ×*Microcitronella* sp. (CRC 1466). None of the test populations from these seed sources were avoided by all three *D. citri* life stages. Seedlings of *Eremocitrus glauca*, *M. inodora*, *M. australasica*, and the *M.* hybrid seed-source genotype were avoided by adults as food and resting hosts, but the *M.* hybrid test population was in the most susceptible egg group, *E. glauca*; *M. inodora* test populations had a moderately high rank for nymphs; and the *M. australasica* test population had a moderately high rank for eggs. Similarly, low numbers of nymphs were found on *S. buxifolia* seedlings, but this test population showed moderate affinity for *D. citri* adults and eggs. Although members of the genus *Fortunella* were received as seed from the Citrus Variety Collection, poor germination prevented their inclusion in the planting; however, two *Fortunella* hybrid test populations in the planting (seedlings of ×*Citrofortunella* sp. and ×*Microcitronella* sp.) showed susceptibility to all life stages of *D. citri* with high mean ranks for eggs in particular.

Seedling test populations of *Glycosmis pentaphylla* and *Clausena harmandiana*, members of Swingle's "remote citroid fruits," also were colonized less by all three life stages of *D. citri*. These species are sexually incompatible with the genus *Citrus*, preventing the creation of resistant intergeneric crosses through sexual

Table 3. Test populations colonized most by each life stage of *D. citri* among seedlings of 87 seed-source genotypes of *Citrus* and *Citrus* relatives surveyed in Ft. Pierce, FL.[z]

Botanical name of seed parent	Common name of seed parent	CRC	Mean rank	Mean count (0–3)
Adults				
Citrus reticulata Blanco	Tien Chieh mandarin	2590	446.4	1.11
Bergera koenigii L.	Curry tree	3165	427.3	1.35
Murraya paniculata (L.) Jack	Orange Jessamine	1637	399.9	1.07
Citrus maxima (Burm.) Merr.	Mato Buntan pummelo	3945	386.8	0.90
Citrus medica L.	Diamante citron	3523	376.7	0.97
Citrus macrophylla Wester	Alemow	3842	375.8	0.83
Nymphs				
×*Citroncirus* sp. (*C. paradisi* 'Duncan' × *P. trifoliata*)	Swingle citrumelo	3771	427.2	2.09
Citrus medica L.	South Coast Field Station citron	3546	425.7	2.07
Citrus taiwanica Tan. & Shimada	Nansho Daidai sour orange	2588	425.0	2.07
Citrus aurantifolia (Christm.) Swing.	Mexican lime type	3822	402.2	1.93
Citrus limettioides Tan.	Palestine sweet lime	1482	400.4	1.88
Citrus limonia (L.) Osbeck	Santa Barbara red lime	712	398.7	1.94
Citrus limonia (L.) Osbeck	Lamas rangpur lime	3919	393.1	1.89
Citrus limon (L.) Burm.*f.*	Local variety from Iran	3885	387.0	1.81
Bergera koenigii L.	Curry tree	3165	386.9	1.81
Citrus paradisi Macf.	Tahitian pummelo × Star Ruby	3781	385.4	1.89
Citrus medica L.	Diamante citron	3523	384.5	1.81
Citrus hassaku, hort ex Tan.	Hassaku pummelo hybrid	3942	381.7	1.83
Citrus aurantifolia (Christm.) Swing.	India lime	2450	379.0	1.79
Citrus neo-aurantium (*C. obovoidea* + *C. unshiu* graft chimera)	Kinkoji Unshiu graft chimera	3816	377.2	1.75
Citrus hassaku hort ex Tan.	Hassaku pummelo hybrid	3907	374.9	1.77
Citrus macrophylla Wester	Alemow	3842	373.1	1.72
Citrus limon (L.) Burm.*f.*	Frost nucellar Lisbon	3176	370.9	1.73
Citrus volkameriana/C. limonia Osbeck	Volkamer lemon hybrid	3050	369.9	1.69
Citrus aurantium L.	Olivelands sour orange	2717	366.4	1.72
Citrus limon (L.) Burm.*f*	Interdonato lemon	3593	365.1	1.66
Citrus reticulata Blanco	Soh Niamtra mandarin	3260	364.9	1.65
Citrus maxima (Burm.) Merr.	Kao Pan pummelo	2242	361.7	1.67
Citrus maxima (Burm.) Merr.	Pomelit pummelo hybrid	4026	361.5	1.72
Citrus reticulata Blanco	Koster mandarin	3958	361.1	1.72
Citrus davaoensis (Wester) Tan.	Davao lemon (Papeda)	2427	361.0	1.70
Citrus maxima (Burm.) Merr.	Kao Panne pummelo	2248	359.5	1.75
Citrus webberi Wester	Kalpi papeda	1455	358.1	1.66
Citrus reticulata Blanco	Som Keowan mandarin	3752	357.4	1.57
Citrus reticulata Blanco	Sun Chu Sha mandarin	4003	352.8	1.62
Eggs				
Citrus taiwanica Tan. & Shimada	Nansho Daidai sour orange	2588	392.4	1.34
Afraegle paniculata (Schum.) Engl.	Nigerian powder flask fruit	297	391.3	1.45
Citrus macrophylla Wester	Alemow	3842	386.5	1.48
Murraya paniculata (L.) Jack	Orange Jessamine	1637	368.6	1.17
Microcitrus hybrid (*M. australis* × *M. australasica*)	Sydney Hybrid	1485	368.3	1.26
Citrus limonia (L.) Osbeck	Santa Barbara red lime	712	365.9	1.26
Bergera koenigii L.	Curry tree	3165	358.5	1.29
Citrus maxima (Burm.) Merr.	Egami Buntan pummelo	3959	352.3	1.20
Citrus neo-aurantium (*C. obovoidea* + *C. unshi* graft chimera)	Kinkoji Unshiu graft chimera	3816	352.2	1.18
Citrus reticulata Blanco	Tien Chieh mandarin	2590	352.1	1.11
Citrus reticulata Blanco	Som Keowan mandarin	3752	346.7	1.00
Murraya paniculata L.	var. ovatifoliolata	3171	345.8	1.03
Citrus limon (L.) Burm.*f.*	Frost nucellar Lisbon	3176	345.2	1.08
Citrus maxima (Burm.) Merr.	Mato Buntan pummelo	3945	343.0	1.19
Citrus reticulata Blanco	Sun Chu Sha mandarin	4003	341.5	1.03
Citrus maxima (Burm.) Merr.	Reinking pummelo	3805	338.3	1.07
Citrus lycopersicaformis hort ex Tan.	Monkey orange	3564	338.3	1.04
Citrus hassaku, hort ex Tan.	Hassaku pummelo hybrid	3942	338.2	1.10
Citrus hybrid (53-1-16 'Clem' × 'Hamlin') × *Chinotto* F1	Sour orange hybrid ex-India	3715	336.7	1.08
Citrus amblycarpa Och.	Nasnaran mandarin	2485	336.7	1.03
Citrus aurantium L.	Gou Tou Cheng	3929	336.4	1.03
Citrus limonia L. Osbeck	Lamas rangpur lime	3919	334.1	1.11
Citrus limon (L.) Burm.*f.*	Local variety from Iran	3885	333.0	1.04
Citrus longispina Wester	Talamisan	2320	332.4	0.96
Citrus reticulata Blanco	Koster mandarin	3958	331.0	1.03
Citrus reticulata Blanco	Scarlet Emperor mandarin Pankan	3326	329.7	1.08

(*Continued on next page*)

hybridization. However, these species may be a source of resistant traits that could be used through genetic transformation. *Glycosmis pentaphylla* is partially graft-compatible with some citrus species (Bowman et al., 2001), and the biochemical basis of resistance in *G. pentaphylla* to a citrus weevil pest, *Diaprepes abbreviatus* (L.) (Shapiro et al., 1997, 2000), might offer some cross-resistance to other insects pests such as *D. citri*.

The majority of the test populations in this study did not exhibit resistance to *D. citri* based on infestation levels we observed, confirming that *D. citri* has a large host range among the Aurantioideae with some of the most susceptible test populations derived from seed sources outside the *Citrus* genus. Even seedlings of *Z. ailanthoides*, one of the two members surveyed in the subfamily Toddalioideae, appeared to be a suitable feeding and resting host for adult *D. citri*. It would be worthwhile to investigate the susceptibility of additional non-Aurantioidea taxa within the Rutaceae to broaden the search for resistant traits and document the true breadth of the *D. citri* host range. With respect to adult susceptibility, the following seed sources provided test populations with the highest adult mean ranks: *C. reticulata* (CRC 2590), *B. koenigii*, *M. paniculata* (1637), *C. maxima* (CRC 3945), *C. medica* (CRC 3523), and *C. macrophylla*. One test population of a *Citrus reticulata* seed source (CRC 2590), 'Tien Chieh', was also in the highest susceptibility group for *D. citri* eggs. This was one of 13 *C. reticulata* selections among the 87 seed sources providing test populations, and although it produced the only *C. reticulata* test population in the highest adult susceptibility group, five other test populations with *C. reticulata* seed sources were identified as susceptible to infestations of eggs and nymphs (Table 3) with three of the *C. reticulata* seed sources providing test populations in both the highest nymph and egg susceptibility groups: 'Soh Niamtra' (CRC 3260), 'Koster' (CRC 3958), and 'Som Keowan' (CRC 3752) mandarins.

Seedlings of *Citrus macrophylla* (alemow) and *B. koenigii* (curry leaf tree) were the only two test populations that were in the highest susceptibility groups for all three life stages, and both are used in research facilities as host plants for laboratory colonies of *D. citri* (Tsagkarakis and Rogers, 2010). Interestingly, *B. koenigii* is a sister taxon to the genus *Clausena* (Bayer et al., 2009), yet its seedlings were heavily colonized by *D. citri* in our studies, whereas seedlings of *C. harmandiana* were not, suggesting that phylogenetic distance among Aurantioideae genotypes may have no influence on *D. citri* susceptibility. *Murraya paniculata* (represented by CRC 1637 in our study), "orange jasmine," is known as second only to commercial citrus as a preferred host for *D. citri* in China (Yang et al., 2006), and this genotype has also frequently been used as a host plant for *D. citri* colonies (Wenninger and Hall, 2007). The recognition of this test population as highly susceptible to *D. citri* was therefore not surprising. Because of orange jasmine's wide use as an ornamental in many

Table 3. (*Continued*) Test populations colonized most by each life stage of *D. citri* among seedlings of 87 seed-source genotypes of *Citrus* and *Citrus* relatives surveyed in Ft. Pierce, FL.[z]

Botanical name of seed parent	Common name of seed parent	CRC	Mean rank	Mean count (0–3)
×*Citroncirus* sp. [*Cleopatra mandarin* ×*trifoliate* (X639)]	X639 trifoliate hybrid	3957	328.3	0.97
Citrus aurantium L.	Zhuluan sour orange hybrid	3930	328.3	0.96
Citrus limon (L.) Burm. *f.*	Mesero lemon	3892	327.1	0.93
Citrus maxima (Burm.) Merr.	Kao Pan pummelo	2242	326.8	1.15
Citrus excelsa Wester	Limon Real	2317	322.2	0.96
Citrus reticulata Blanco	Soh niamtra mandarin	3260	319.3	0.90
×*Citrofortunella* sp.	Tavares limequat	3172	318.4	0.86
Citrus maxima (Burm.) Merr.	Kao Panne pummelo	2248	318.0	1.00
Citrus medica L.	South Coast Field Station citron	3546	317.5	1.00
Citrus limon L. Burm.*f.*	Frost nucellar Eureka lemon	3005	317.3	1.08
Citrus davaoensis (Wester) Tan.	Davao lemon (Papeda)	2427	315.9	0.93
×*Citroncirus* sp.	S-281 Citrangelo	3552	314.9	0.83
Citrus intermedia hort ex Tan.	Yama-mikan sour orange	3474	314.5	0.93
Citrus maxima (Burm.) Merr.	Pomelit Pummelo hybrid	4026	313.2	0.97
×*Microcitronella* sp. (*M. australasica* × Calamondin).	Faustrimedin	1466	311.3	0.96

[z]For each life stage, seed-source genotypes are listed in order of decreasing colonization of their seedling test populations. Members of the Rutaceae vary greatly in their incidence of nucellar embryony (reviewed in Frost and Soost, 1968) and so some of the plants tested were essentially genetically identical to the seed parent, whereas others represent half-sib families with only the seed parent known.

parts of the world, there is significant interest in the role of this host plant in the maintenance and possible amplification of HLB (Damsteegt et al., 2010; Lopes et al., 2010). There is some controversy over whether the "orange jasmine" commonly found as a host for *D. citri* in Florida and Brazil is *M. paniculata* or *M. exotica*, because the two names at times have been used interchangeably, but recent molecular evidence suggests that *M. exotica* can be treated as a synonym for *M. paniculata* and may possibly represent *M. paniculata* var. *ovatifoliolata* (Ranade et al., 2006). In the current study, two test populations of *M. paniculata* were surveyed. Seedlings of *Murraya paniculata* identified by the CRC number 1637 was in the highest groups for the presence of adults and eggs, and according to the Citrus Variety Collection, the plant from which seeds for this study were derived was received as a live plant from W.T. Swingle in 1926. The *Murraya paniculata* associated with the CRC number 3171, which was in the highest test population group for egg presence, is according to the Citrus Variety Collection probably var. *ovatifoliolata* and the parent plant was germinated from seed received by the Horticulture Department at the University of Hawaii Agriculture Experiment Station in 1955.

Multiple seed-source genotypes of several other *Citrus* species provided multiple test populations that were found to be highly susceptible to *D. citri*, including *C. maxima* (pummelo), *C. medica* (citron), and *C. limonia* (rangpur lime). All six pummelo test populations surveyed—from the seed sources 'Egami Buntan' (CRC 3959), 'Mato Buntan' (CRC 3945), 'Reinking' (CRC 3805), 'Kao Pan' (CRC 2242), 'Kao Panne' (CRC 2248), and 'Pomelit' (CRC 4026)—were in the highest group for egg susceptibility. Two of the three different citron test populations ['Diamante'(CRC 3523) and 'South Coast Field Station' (CRC 3546) seed sources] and each of two test populations of rangpur limes ['Santa Barbara red' (CRC 712) and 'Lamas' (CRC 3919) seed sources] were in two of three life stage highest susceptibility groups.

How plant material with mixed susceptibility to different life stages of *D. citri* may influence the overall epidemiology of HLB is difficult to interpret. For HLB transmission, adults represent a key life stage because their mobility allows them to acquire the pathogen and then inoculate subsequent host plants. Plants that may be used by adults for feeding, but infrequently used for oviposition and subsequent nymphal development, may still act as an important source of infection for immigrating adults. However, a recent study reported that in laboratory studies, nymphs reared on HLB-infected plants were more likely to acquire the bacterium than adults (Pelz-Stelinski et al., 2010). Therefore, an infected plant that is host to large numbers of developing nymphs may be a more important source of infected adults. How the pathogen manifests in different host plant genotypes is also a key element in predicting how the disease moves through an area.

Genotypes that express a level of resistance against infestation by *D. citri* may be using biochemical and/or behavioral mechanisms. Some genotypes highly attractive to ovipositing females (and thus highly susceptible to becoming infested by adults and eggs) may contain traits resistant to nymphs. There are multiple examples in nature of negative correlations between oviposition preference and offspring performance (Thompson, 1988; Wise et al., 2008). Plant material that is attractive to ovipositing females but contains compounds that adversely affect developing nymphs may have value in reducing the population equilibrium of *D. citri* in a grove. It is also possible that a lack in correspondence in susceptibility among the life stages in a test population may have been influenced by sampling date. Adults are mobile and eggs are only in the field for ≈4 days at 25 °C before hatching; thus, the presence and abundance of these two life stages are more difficult to reliably document. On the other hand, surviving nymphs may be on a plant for 12 or more days depending on temperature, host plant, and other factors (Tsai and Liu, 2000).

A strength of this study was the ability to screen seedlings derived from numerous diverse genotypes in the field that were exposed to a natural population of *D. citri* rather than relying on cage or greenhouse experiments with colony-reared *D. citri*. However, in attempts to sample all replicates in a reasonable time period, categorical counts were used as a compromise between merely recording presence and counting large numbers of eggs, nymphs, and adults on each replicate. Because the primary purpose of this survey was to identify potential sources of resistance, categorical counts were adequate for this level of discernment. In future studies that attempt to identify more subtle differences in susceptibility, full counts may identify smaller genotype effects.

In summary, the work described here has identified a few genotypes providing seedlings expressing either complete or high levels of resistance to *D. citri* based on reduced infestation levels of the psyllid in a free-choice situation. It has also defined the relative level of susceptibility to the three life stages of *D. citri* among seedlings of many *Citrus* species and more distant members of the Aurantioideae subfamily. Because of its importance in citrus breeding programs, future work will include an in-depth investigation into probable resistance in *P. trifoliata*. Probable resistance in other genotypes avoided by *D. citri* should be explored, and an expanded survey within the Aurantioideae and other Rutaceae genera not represented in the study presented here should be carried out.

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