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Further investigations on colonization of *Poncirus trifoliata* by the Asian citrus psyllid

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A B S T R A C T

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) vectors a bacterium, '*Candidatus* Liberibacter asiaticus' (CLas), associated with one of the world's most serious diseases of citrus, huanglongbing (HLB) (also known as citrus greening disease). There is no known cure for this disease, which severely reduces tree productivity and fruit quality and promotes tree decline and death. Most growers apply insecticides to control ACP as a tactic to prevent or reduce the incidence of HLB. Plant resistance to ACP may be a viable tactic for managing ACP and CLas. Resistance to colonization by ACP populations has been reported in the genotype *Poncirus trifoliata* L., which is cross compatible with citrus. The Citrus Research Center (CRC) in Riverside, California, maintains a large number of accessions of *P. trifoliata*. We conducted four free-choice experiments with 29 CRC accessions of *P. trifoliata* and found that ACP deposited fewer eggs on 19 of those accessions compared with susceptible sweet orange, *Citrus sinensis* (L.) Osbeck. Subsequent infestation densities of nymphs generally reflected reductions in oviposition but there were notable exceptions. Some accessions may be susceptible to oviposition but contain traits that confer antibiosis to nymphs. In no-choice experiments with seedling plants representing eight *P. trifoliata* accessions, oviposition rates were reduced compared with sweet orange and it appeared that factors associated with mature leaves were involved. In no-choice experiments with adult ACP confined to flush cuttings, large reductions were observed in the number of eggs and nymphs on each of five *P. trifoliata* accessions compared with sweet orange. Based on these results in conjunction with published reports, accessions were identified that consistently showed resistance to oviposition. However, the level of resistance in these accessions has been variable possibly due to environmental conditions, plant age, proximity of plants to susceptible germplasm, and other factors.

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1. Introduction

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, is an important pest of citrus because it vectors a bacterium considered responsible for a serious bacterial disease of citrus known as Asiatic huanglongbing (HLB) (also known as citrus greening disease) (Bové, 2006; Gottwald, 2010). The bacterium, '*Candidatus* Liberibacter asiaticus', causes trees to become unthrifty and reduces yield and crop quality. Juice from infected trees develops a bitter taste; infected trees decline and can die, especially young trees (Halbert and Manjunath, 2004; Grafton-Cardwell et al., 2013; Hall et al., 2013a). It is difficult to get new citrus plantings into production before trees succumb to the disease (Hall et al., 2013b). The origin of ACP and CLas is thought to be southwestern Asia, but the vector and disease have spread to other citrus-producing areas around the world including the Americas. The disease is currently devastating citrus production in Florida in the United States, where the future of citrus remains unclear.

Following the 2005 discovery of HLB in Florida, experts recommended that growers follow a three-component disease management program: plant only disease-free trees, find and remove infected trees as quickly as possible, and establish an intensive insecticide program against the psyllid (Hall et al., 2013a). However, this program has not been viewed as sustainable due to high costs, grower reluctance to remove infected trees that are still productive, and negative effects of insecticides on non-targets (Hall and Gottwald, 2011). Additionally, insecticides can be relatively ineffective for preventing introduction and spread of the disease organism in new plantings (Hall et al., 2013b). Alternative ACP/HLB

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management strategies continue to be sought, and host plant resistance to ACP is one tactic of interest.

Some general information on ACP host range has been published (Halbert and Manjunath, 2004; Peña et al., 2006). A vast majority of the plant species utilized by ACP as reproductive hosts fall within the family Rutaceae, subfamily Aurantioideae (citrus subfamily) (Hall et al., 2013a). This subfamily consists of two Tribes, the Aurantieae (which includes three subtribes of genera, many of which are utilized by ACP as reproductive hosts including *Citrus* and *Murraya*) and the Clauseneae (four genera including *Bergera*, also a good ACP reproductive host). Significant differences in fecundity, longevity and other life parameters have been reported for ACP reared on *Citrus* and other genera considered to be ACP-susceptible (Tsai and Liu, 2000; Fung and Chen, 2006; Nava et al., 2007). Few studies have been conducted to identify germplasm with resistance to ACP. Tsagkarakis and Rogers (2010) reported that ACP biological fitness was significantly reduced on 'Cleopatra' mandarin (*Citrus reshni* Hort. Ex Tan.) compared with 'sour orange' (*Citrus aurantium* L.). Aubert (1987) noted that *Poncirus trifoliata* L. was colonized less by ACP than other genotypes. Interestingly, *Citrus* and *Poncirus* are placed in the subtribe Citrinae and are cross compatible (Ziegler and Wolfe, 1975). Very low numbers of ACP were observed to colonize two accessions of *P. trifoliata* while large numbers were observed on many accessions of *Citrus* and other genera (Westbrook et al., 2011). Nearly all of 47 tested accessions of *P. trifoliata* and several of 33 ×*Citroncirus* (hybrids of *P. trifoliata* and another parent species) were found to have resistance to colonization by ACP in a no-choice setting (Richardson and Hall, 2013).

With respect to evaluating germplasm for resistance to ACP, the USDA-ARS National Clonal Germplasm Repository for Citrus and Dates (NCGRCD) located at the University of California at Riverside collects, stores and makes available seeds of many genotypes of *Citrus* and *Citrus* relatives. This is accomplished in collaboration with the Citrus Research Center at the University of California at Riverside, which maintains a citrus variety collection of >1000 accessions with unique Citrus Research Center (CRC) numbers. Botanical and common names of source material along with other information for CRC accessions can be found at http://citrusvariety.ucr.edu/citrus/index.html.

The results of three studies on colonization by ACP of different accessions of *P. trifoliata* and ×*Citroncirus* are presented here. The first study consisted of four separate greenhouse experiments in which a combined total of 57 accessions of *P. trifoliata* and ×*Citroncirus* were evaluated for resistance to ACP colonization in a free-choice situation. The second study consisted of four separate experiments in which seedling plants of a combined total of nine accessions (including eight *P. trifoliata* and one ×*Citroncirus*) were evaluated for resistance to ACP oviposition in a no-choice situation. The third study examined oviposition and adult survival on flush cuttings of five *P. trifoliata* accessions.

2. Materials and methods

Seeds were obtained from NCGRCD and planted in individual plastic cells (3.8 cm dia. by 21 cm) (SC-10 super cell Cone-tainers, Stuewe and Sons, Tangent, OR) containing sterile potting mix. Members of the Rutaceae vary greatly in the incidence of nucellar embryony (Frost and Soost, 1968). Therefore, some of the plants tested could have been clones of the seed parent while others could have represented half-sib families with only the seed-parent known. It was possible that sexually-derived seedlings were included in these studies and these could differ in their susceptibility to ACP and inflate data variation.

ACP adults for these studies were obtained from a colony established in 2000 at the USDA-ARS U.S. Horticultural Research Laboratory (Fort Pierce, FL). The psyllids were originally collected from citrus in the field and subsequently reared in a greenhouse in cages containing orange jasmine, *Murraya exotica* L. (= *Murraya paniculata* auct. non.), until March 2010, when *Citrus macrophylla* Wester was substituted as the rearing plant. The colony is maintained by transferring adults to new plants every 14 d using procedures similar to those described by Skelley and Hoy (2004), with no infusion of wild types. The colony is confirmed quarterly by qPRC (Li et al., 2006) to be free of CLas.

2.1. Colonization of *P. trifoliata* in free-choice greenhouse tests

Four free-choice experiments were conducted to assess colonization by ACP of 29 *P. trifoliata* and 28 ×*Citroncirus* accessions. The experiments were conducted in a small air-conditioned greenhouse (Conley's Hobby House JS1100-B, Montclair, CA) at different times of the year with associated differences in temperature and photoperiod, thus direct comparisons of ACP populations among the experiments were not made. The first experiment (August 2013) assessed 20 accessions (15 *P. trifoliata* and 5 ×*Citroncirus*); the second (September 2013) included 19 accessions (12 *P. trifoliata* and 7 ×*Citroncirus*); the third (November 2013) included 8 accessions (1 *P. trifoliata* and 7 ×*Citroncirus*); and the fourth (March 2014) included 12 accessions (1 *P. trifoliata* and 11 ×*Citroncirus*). The four experiments consisted of five replications (plants) of each accession and a susceptible check *Citrus sinensis* (L.) Osbeck 'Ridge Pineapple'. One-to two-year-old plants of a similar size growing in identical pots were arranged in a randomized complete block design in racks on greenhouse benches. The racks were specifically for the SC-10 Cone-tainers, and in each experiment one rack was used for each replication. Plants were trimmed to stimulate regrowth (flush). Adult ACP were released into the greenhouse and allowed to disperse and select host plants. Depending upon numbers available, approximately 4700, 3400, 6100, and 6000 ACP of unknown ages were released during the first several days at the beginning of experiments 1, 2, 3 and 4, respectively. Each plant was inspected for eggs and nymphs weekly for three weeks. Egg and nymph abundances were recorded as categorical counts based on a 0 to 3 semi-quantitative scale (Westbrook et al., 2011). Egg sample categories per flush shoot were 0 = 0; 1 = 1 to 20; 2 = 21 to 40; and 3 = >40 eggs. Nymph sample categories per flush shoot were 0 = 0; 1 = 1 to 10; 2 = 11 to 30; and 3 = >30 nymphs. Four flush shoots were randomly examined on each plant (sometimes fewer if four were not present). Mean ratings for egg and nymph densities per flush shoot for each plant on each sample date were computed and subjected to a generalized linear mixed model using PROC GLIMMIX in SAS (SAS Institute, 2010) and compared by least squares means (LSMEANS option in SAS). The analyses were first conducted on data from all flush shoots observed including those not infested, and then the analyses were repeated but only on data for infested flush shoots. All statistical tests were conducted at the 0.05 level of significance.

2.2. Oviposition on *P. trifoliata* seedlings in a no-choice situation

Four experiments were conducted to assess ACP oviposition on eight to nine month-old *P. trifoliata*. The first experiment included three *P. trifoliata* accessions and one ×*Citroncirus* accession; the second experiment included five *P. trifoliata* accessions; the third experiment included the same accessions as the first experiment; and the fourth experiment included the same accessions as the second experiment. 'Ridge Pineapple' was included in each experiment as an ACP-susceptible check plant. For the first two experiments, we completely removed the leaves from each of 12 seedlings of each accession to stimulate flush. For the third and

Table 1
Colonization of seedling test populations of *Poncirus trifoliata* and ×*Citroncirus* by *Diaphorina citri* surveyed in Ft. Pierce, FL, August 2013. Four flush shoots were examined for eggs and nymphs on five plants of each genotype weekly for three weeks. Data are listed in order of decreasing mean egg counts per flush shoot (over all shoots observed). Mean daily temperature 29.3 ± 0.9 °C, mean minimum 24.8 ± 0.6 °C, and mean maximum 38.3 ± 2.1 °C.

Botanical name of seed parent (parentage of hybrids) (CRC[b])	Common name of seed parent	Mean density rating per shoot over all flush shoots[a]		Mean density rating per shoot among infested flush shoots[a]	
		Eggs	Nymphs	Eggs	Nymphs
×*Citroncirus* sp. (*P. trifoliata* × *C. sinensis*) (2748)	Morton	1.50a	1.83a	2.12abc	2.73ab
×*Citroncirus* sp. (*P. trifoliata* × *C. limon*) (1449)	Citremon	1.37ab	1.41abcd	2.07abc	2.45abcd
Citrus sinensis (–)	Ridge pineapple	1.33abc	1.88a	2.35ab	2.99a
Poncirus trifoliata L. (3213)	Kryder 60-2	1.18abcd	1.48abc	1.92abcde	2.43abcde
Poncirus trifoliata L. (3211)	Rich 22-2	1.17abcd	1.56ab	2.47a	2.70abc
×*Citroncirus* sp. (*P. trifoliata* × *C. sinensis*) (301)	Rusk	1.10abcd	1.38abcd	1.81bcde	2.31abcde
×*Citroncirus* sp. (*P. trifoliata* × *Citrus* sp) (2865)	Uvalde	1.03abcde	1.40abcd	1.78bcde	2.25abcdef
×*Citroncirus* sp. (*P. trifoliata* × *C. sinensis*) (271)	Cunningham	0.86bcdef	0.91defgh	2.31ab	2.55abcd
Poncirus trifoliata L. (2862)	Florida	0.83bcdef	1.22bcde	1.73bcde	2.25abcdef
Poncirus trifoliata L. (1498)	USDA	0.80cdef	0.98cdefg	2.00abcde	2.11bcdef
Poncirus trifoliata L. (2554)	Barnes	0.65defg	1.01cdefg	1.59cde	2.44abcde
Poncirus trifoliata L. (3219)	Kryder 28-3	0.54efg	1.14bcdef	1.82abcde	2.20abcdef
Poncirus trifoliata L. (3548)	English	0.50efg	0.83efgh	1.57cde	1.91cdef
Poncirus trifoliata L. (3218)	Kryder 8-5	0.46fg	0.63fgh	1.62cde	1.59f
Poncirus trifoliata L. (3212)	Kryder Medium	0.46fg	0.93defgh	1.74bcde	2.10bcdef
Poncirus trifoliata L. (3210)	Kryder 16-6	0.43fg	0.66fgh	1.34e	1.73def
Poncirus trifoliata L. (838)	Rubidoux	0.38fg	1.03bcdefg	1.50cde	1.63f
Poncirus trifoliata L. (1717)	Pomeroy	0.38fg	0.52gh	2.04abcd	1.74def
Poncirus trifoliata L. (3215)	Kryder 55-5	0.37fg	0.56gh	2.04abcd	2.16abcdef
Poncirus trifoliata L. (3206)	Argentina	0.31fg	0.53gh	1.42de	1.85cdef
Poncirus trifoliata L. (2552)	Webber-Fawcett#22	0.18g	0.42h	1.68bcde	1.43f

[a] A rating scale of the 0–3 was used to estimate egg and nymph densities per flush shoot, details are provided in the text. Means in the same column followed by the same letter are not significantly different ($P = 0.05$), least squares means.

[b] Citrus Research Center, Riverside, California accession number.

fourth experiments, ~2.5 cm of the top of each plant was clipped to stimulate flush while leaving mature leaves on each plant. In each experiment, we selected six plants of each accession at ten days after trimming and removed all but two flush shoots. Three ACP females and two males (~15 days old) from a colony maintained on orange jasmine in an air-conditioned greenhouse were placed on each plant. A plastic, ventilated cylinder (37 mm dia. × 305 mm) (Richardson and Hall, 2013) was placed over each plant to confine the psyllids. The open bottom of each cylindrical cage was pressed down into the Cone-tainer to secure the cage. The plants were arranged in a random complete block design in racks and placed under lights on a laboratory bench (23 °C, 53% relative humidity,

Table 2
Colonization of seedling test populations of *Poncirus trifoliata* and ×*Citroncirus* by *Diaphorina citri* surveyed in Ft. Pierce, FL, September 2013. Four flush shoots were examined for eggs and nymphs on five plants of each genotype weekly for three weeks. Data are listed in order of decreasing mean egg counts per flush shoot (over all shoots observed). Mean daily temperature 28.4 ± 0.3 °C, mean minimum 24.8 ± 0.1 °C, and mean maximum 39.4 ± 0.8 °C.

Botanical name of seed parent (parentage of hybrids) (CRC[b])	Common name of seed parent	Mean density rating per shoot over all flush shoots[a]		Mean density rating per shoot among infested flush shoots[a]	
		Eggs	Nymphs	Eggs	Nymphs
Citrus sinensis (–)	Ridge pineapple	1.95a	1.62a	2.43ab	2.51a
×*Citroncirus* sp. (*C. paradisi* × *P. trifoliata* (3821)	Citrumelo	1.45ab	0.46bcde	2.75a	1.72bcd
×*Citroncirus* sp. (*P. trifoliata* × *C. paradisi*) (1452)	Citrumelo	1.42b	0.59bcd	2.29abc	1.57bcd
×*Citroncirus* sp. (*P. trifoliata* × *C. sinensis*) (3908)	Benton	1.30b	0.62bc	2.12bcd	1.84abc
×*Citroncirus* sp. (‘Willits citrange’ x *C. madurensis*) (3573)	Glen Citrangedin	1.20bc	0.35bcde	1.93bcdef	1.30bcd
×*Citroncirus* sp. ((*P. trifoliata* × *C. sinensis*) x *C. unshiu*) (3415)	S-302 Citranguma	1.19bc	0.46bcde	2.72a	1.63bcd
×*Citroncirus* sp. (unknown parentage) (2592)	Calamondin	1.13bcd	0.58bcd	2.06bcd	1.82bc
×*Citroncirus* sp. (*C. sinensis* × *P. trifoliata* (3336)	Spanish	1.11bcde	0.74b	1.99bcde	1.57bcd
Poncirus trifoliata L. (3571)	Taylor	0.78cdef	0.46bcde	1.73bcdefg	1.32bcd
Poncirus trifoliata L. (3586)	Kryder 5-5	0.77cdef	0.27cde	1.74bcdefg	1.40bcd
Poncirus trifoliata L. (3587)	Rich 7-5	0.67defg	0.15e	1.51defg	1.42bcd
Poncirus trifoliata L. (3882)	Hiryu	0.61efg	0.58bcd	1.53defg	1.49bcd
Poncirus trifoliata L. (3547)	Benoit	0.58fg	0.24cde	1.46defg	1.59bcd
Poncirus trifoliata L. (3588)	Marks	0.49fg	0.19de	1.17g	1.36bcd
Poncirus trifoliata L. (3209)	Rich 12-2	0.48fg	0.19de	1.34fg	1.17cd
Poncirus trifoliata L. (3570)	Ronnse	0.44fg	0.31cde	1.32fg	1.23cd
Poncirus trifoliata L. (3412)	Yamaguchi	0.38fg	0.24cde	1.35efg	1.09d
Poncirus trifoliata L. (3338)	Benecke	0.37fg	0.23cde	1.66cdefg	1.29bc
Poncirus trifoliata L. (3939)	#26	0.27fg	0.29cde	1.71bcdefg	1.92ab
Poncirus trifoliata L. (3486)	Kryder 55-1	0.21g	0.31cde	0.98g	1.71bcd

[a] A rating scale of the 0–3 was used to estimate egg and nymph densities per flush shoot, details are provided in the text. Means in the same column followed by the same letter are not significantly different ($P = 0.05$), least squares means.

[b] Citrus Research Center, Riverside, California accession number.

Table 3
Colonization of seedling test populations of *Poncirus trifoliata* and ×*Citroncirus* by *Diaphorina citri* surveyed in Ft. Pierce, FL, November 2013. Four flush shoots were examined for eggs and nymphs on five plants of each genotype weekly for three weeks. Data are listed in order of decreasing mean egg counts per flush shoot (over all shoots observed). Mean daily temperature 25.2 ± 0.1 °C, mean minimum 23.8 ± 0.1 °C, and mean maximum 29.0 ± 0.6 °C.

Botanical name of seed parent (parentage of hybrids) (CRC[b])	Common name of seed parent	Mean density rating per shoot over all flush shoots[a]		Mean density rating per shoot among infested flush shoots[a]	
		Eggs	Nymphs	Eggs	Nymphs
×*Citroncirus* sp. (*C. maxima* × *P. trifoliata*) (3969)	African Shaddock × Rubidoux	1.39a	1.28ab	1.97ab	2.12ab
×*Citroncirus* sp. (*P. trifoliata* × *C. limon*) (1448)	Citremon	1.32a	0.78bc	2.00ab	1.05c
Citrus sinensis (−)	Ridge pineapple	1.29a	1.58a	1.67ab	2.38a
×*Citroncirus* sp. ('Rangpur' lime × Troyer) (3997)	Rangpur × Troyer	1.23a	1.23ab	2.18ab	1.61abc
×*Citroncirus* sp. (*P. trifoliata* × *C. sinensis*) (275)	Savage	1.07ab	1.07abc	2.72a	2.01ab
×*Citroncirus* sp. (*P. trifoliata* × *C. aurantium*) (1438)	Citradia	0.96abc	1.22ab	2.29ab	0.91c
×*Citroncirus* sp. (*P. trifoliata* × *C. sinensis*) (276)	Sanford	0.84abc	1.11abc	0.96b	1.17bc
×*Citroncirus* sp. (*P. trifoliata* × *C. reticulata*) (2618)	Citrandarin	0.51bc	0.44cd	2.27ab	2.55a
Poncirus trifoliata L. (3572)	Towne "F"	0.11c	0.13d	2.3a	1.07c

[a] A rating scale of the 0–3 was used to estimate egg and nymph densities per flush shoot, details are provided in the text. Means in the same column followed by the same letter are not significantly different ($P = 0.05$), least squares means.
[b] Citrus Research Center, Riverside, California accession number.

14 h daily illumination). Adult ACP were introduced on September 31, August 12, September 15, and October 6 in experiments 1, 2, 3 and 4, respectively. Eggs were counted four days after ACP were introduced in the first and third experiments and after two days in the second and fourth experiments. We shortened the oviposition period in these two experiments because visual observations indicated substantial numbers of eggs were present after two days. The number of eggs on each flush shoot was counted under a dissection microscope. Counts were summed for a total number of eggs per plant, and the total number was subsequently converted to number of eggs per female per day. The egg-count data were subjected to an analysis of variance (PROC ANOVA procedure in SAS) and Tukey's studentized range (HRD) test was used for comparisons of means. All statistical tests were conducted at the 0.05 level of significance.

2.3. Oviposition behavior and survival on *P. trifoliata* flush cuttings

Oviposition behavior and survival of ACP on flush cuttings of *P. trifoliata* were studied. The plants for the study were seeded and grown in a greenhouse and were about six months old when the experiment was initiated during late August 2014. A flush cutting was a small branch or the central stem (12–15 cm in length) of a seedling with 3 or 4 mature leaves and 1 or 2 young flush shoots. Flush cuttings were excised from plants using clippers and washed in distilled water (DI). The cut end of each flush cutting was slipped into a glass vial filled with DI water and secured to the vial using Parafilm 'M' laboratory film (American National Can, Chicago, IL), which also prevented evaporation of water. The following CRC accessions of *P. trifoliata* were studied: 'Kryder 55-5' (3215), 'Towne-G' (3207), 'Simmons' (3549), 'Yamaguchi' (3412) and 'Hiryu' (3882). *C. macrophylla* and orange jasmine were included as control plants. A flush cutting from each plant was placed into a 30 × 30 × 30 cm mesh cage (#1466ASV, BioQuip, San Diego, CA), after which 25 adult ACP were introduced into the cage (6 replications) and allowed to feed and oviposit for 14 days. Cages were kept in an incubator (25 °C, 75% relative humidity, 14 h daily illumination). All ACP used in the study were 10–12 d-old. Two groups of ACP were studied: ACP taken directly from a colony maintained on *C. macrophylla*, and ACP from the same colony moved to *M. exotica* for 4 days before being introduced into cages. The two ACP groups were studied to provide insight into oviposition by ACP transferred

Table 4
Colonization of seedling test populations of *Poncirus trifoliata* and ×*Citroncirus* by *Diaphorina citri* surveyed in Ft. Pierce, FL, March 2014. Four flush shoots were examined for eggs and nymphs on five plants of each genotype weekly for three weeks. Data are listed in order of decreasing mean egg counts per flush shoot (over all shoots observed). Mean daily temperature 28.2 ± 0.3 °C, mean minimum 23.6 ± 0.2 °C, and mean maximum 40.1 ± 1.0 °C.

Botanical name of seed parent (parentage of hybrids) (CRC[b])	Common name of seed parent	Mean density rating per shoot over all flush shoots[a]		Mean density rating per shoot among infested flush shoots[a]	
		Eggs	Nymphs	Eggs	Nymphs
×*Citroncirus* sp. (*C. paradisi* × *P. trifoliata*) (3889)	C-190	1.60a	2.15ab	2.78ab	2.45ab
Citrus sinensis (−)	Ridge pineapple	1.57a	2.15ab	2.96ab	2.99a
×*Citroncirus* sp. (African shaddock × Rubidoux) (3969)	–	1.56a	2.22ab	2.90ab	2.98a
×*Citroncirus* sp. ((*P. trifoliata* × *C. sinensis*) x *C. sinensis*) (1447)	Citrangor	1.48a	2.03ab	2.97a	2.68ab
×*Citroncirus* sp. (*C. sinensis* × *P. trifoliata*) (3911)	C-32	1.41a	1.70bc	2.84ab	2.62ab
×*Citroncirus* sp. ((*C. paradisi* × *C. tangerina*) x *P. trifoliata*)) (3954)	Trifeola	1.22a	2.20ab	3.00a	2.92a
×*Citroncirus* sp. (*P. trifoliata* × *C. limon*) (1448)	Citremon	1.22a	2.14ab	2.72ab	2.49ab
×*Citroncirus* sp. (*P. trifoliata* × *C. sinensis*) (1463)	Morton	1.15a	2.74a	2.61ab	2.84ab
×*Citroncirus* sp. (*P. trifoliata* × *C aurantium*) (1436)	Citradia	1.04a	2.07ab	2.89ab	2.63ab
×*Citroncirus* sp. (chimaera *P. trifoliata* × *C. unshiu*) (3881)	Citrondarin	1.01ab	2.14ab	2.90ab	2.38ab
×*Citroncirus* sp. (*C. reshni* × *P. trifoliata*) (3957)	X639	0.95ab	2.60a	2.36bc	2.27ab
×*Citroncirus* sp. (*P. trifoliata* x (*C. paradisi* × *C. reticulata*)) (3552)	S-281 Citrangelo	0.81ab	2.53a	2.73ab	2.88ab
Poncirus trifoliata L. (3207)	Towne "G"	0.19b	1.00c	1.83c	2.10b

[a] A rating scale of the 0–3 was used to estimate egg and nymph densities per flush shoot, details are provided in the text. Means in the same column followed by the same letter are not significantly different ($P = 0.05$), least squares means.
[b] Citrus Research Center, Riverside, California accession number.

Table 5
Number of eggs laid by Asian citrus psyllid on different accessions of *Poncirus trifoliata* and one of its hybrids (×*Citroncirus*) compared to sweet orange 'Ridge Pineapple'. Mean ± SEM temperature 23.0 ± 0.1 °C, minimum 22.2 °C, and maximum 23.6 °C.

Oviposition dates	Host plant genotype, CRC[a], and 'common name'	Mean (SEM) number of eggs/female/day[b]
Seedling plants with flush, all mature leaves removed (n = 6)		
7/31–8/4	×*Citroncirus* 2748 'Morton'	26.3 (2.2) a
	Citrus sinensis 'Ridge Pineapple'	17.4 (2.3) b
	Poncirus trifoliata 1717 'Pomeroy'	16.6 (2.4) bc
	Poncirus trifoliata 3572 'Towne F'	14.4 (1.9) bc
	Poncirus trifoliata 3215 'Kryder 55-5'	8.1 (2.0) c
8/12–8/14	*Citrus sinensis* 'Ridge Pineapple'	26.0 (3.7) a
	Poncirus trifoliata 2552 'Webber-Fawcett#22'	17.1 (3.6) ab
	Poncirus trifoliata 838 'Rubidoux'	13.0 (3.5) ab
	Poncirus trifoliata 3549 'Simmons'	12.9 (3.0) ab
	Poncirus trifoliata 3210 'Kryder 16-6'	12.4 (3.9) ab
	Poncirus trifoliata 4172 '#22'	11.3 (2.1) b
Seedling plants with both flush and mature leaves (n = 6)		
9/15–9/19	*Citrus sinensis* 'Ridge Pineapple'	29.8 (3.5) a
	×*Citroncirus* 2748 'Morton'	26.5 (3.4) a
	Poncirus trifoliata 3572 'Towne F'	5.3 (1.8) b
	Poncirus trifoliata 3215 'Kryder 55-5'	5.2 (1.9) b
	Poncirus trifoliata 1717 'Pomeroy'	4.9 (1.8) b
10/6–10/8	*Citrus sinensis* 'Ridge Pineapple'	25.8 (3.6) a
	Poncirus trifoliata 3549 'Simmons'	20.6 (2.9) ab
	Poncirus trifoliata 2552 'Webber-Fawcett#22'	17.5 (2.6) abc
	Poncirus trifoliata 3210 'Kryder 16-6'	13.4 (3.3) abc
	Poncirus trifoliata 4172 '#22'	9.1 (4.1) bc
	Poncirus trifoliata 838 'Rubidoux'	6.0 (3.0) c

[a] Citrus Research Center, Riverside, California accession number (*C. sinensis* seed obtained locally, thus no CRC number).

[b] For each group of plants, means in the same column followed by the same letter are not significantly different ($P = 0.05$), Tukey's studentized range (HRD) test.

between different host plants. There were three replications for each group of ACP. Each flush shoot was examined 14 d after introducing ACP to count numbers of live adults and immatures (eggs and nymphs). Counts were analyzed as a 2 × 7 factorial with major effects of colony (ACP source) and host plant (five accessions of *P. trifoliata* and two controls). Tukey's HSD test was used for comparison of means. All statistical tests in the oviposition trial were conducted at the 0.05 level of significance using Statistix software (Statistix 10, Tallahassee, FL).

3. Results and discussion

3.1. Colonization of *P. trifoliata* in free-choice greenhouse tests

ACP eggs and nymphs were observed on every accession studied during the four experiments (Tables 1–4), but mean density ratings often were significantly lower on many of the *P. trifoliata* accessions compared with the susceptible check (Tables 1 and 2). As reflected by mean densities across all flush shoots observed, many *P. trifoliata* shoots were not colonized at all. Considering all *P. trifoliata* CRC accessions across the four experiments, the following 19 were judged to be resistant to oviposition in a free-choice setting compared with the susceptible control (*C. sinensis*) based on reduced oviposition: CRC 838, 2554, 2862, 3206, 3207, 3209, 3210, 3212, 3218, 3338, 3412, 3486, 3547, 3548, 3570, 3586, 3588, 3587, and 3882. The following 10 accessions did not appear to have any resistance to oviposition compared with the susceptible control: CRC 1498, 1717, 2552, 2862, 3211, 3212, 3213, 3215, 3219, and 3572. None of the 29 ×*Citroncirus* accessions showed reduced oviposition. Westbrook et al. (2011) studied four ×*Citroncirus* accessions including three that we studied (CRCs 301, 3552, and 3957) and reported that each was susceptible to ACP colonization. We observed that infestation densities of nymphs on accessions of *P. trifoliata* generally reflected reductions in oviposition with notable exceptions (e.g., CRC 2554, Table 1). It is possible that ACP readily oviposit on some *P. trifoliata* accessions such as CRC 1717 and 3572 that possess traits that confer antibiotic resistance to nymphs.

Fifty-three *P. trifoliata* and ×*Citroncirus* accessions studied here were included in no-choice experiments by Richardson and Hall (2013). In both studies, oviposition was reduced relative to a susceptible check on 16 of 28 accessions of *P. trifoliata* (57%). In most cases where our results were in general agreement, reductions in oviposition reported by Richardson and Hall (2013) were larger. Of 33 ×*Citroncirus* accessions in that study, 10 were resistant to ACP oviposition in no-choice tests compared with a susceptible check (*C. macrophylla*). We studied 29 ×*Citroncirus* accessions including 26 studied by Richardson and Hall (2013). None of the accessions showed resistance to ACP oviposition in our free-choice tests. Only general comparisons can be made between our study and the one by Richardson and Hall (2013) due to differences in objectives, time of year and experimental procedures including the genotypes used as controls.

3.2. Oviposition on *P. trifoliata* seedlings in a no-choice situation

In the first experiment, significantly fewer eggs per female per day were observed on *P. trifoliata* accession 3215 than on the susceptible check 'Ridge Pineapple', but no significant differences in oviposition rates were observed between the check and either of two other accessions, 1717 and 3572 (Table 5). These results were in general agreement with those reported by Richardson and Hall (2013) for accessions 3572 (not resistant to oviposition) and 3215 (resistant to oviposition). Oviposition was significantly greater on the ×*Citroncirus* accession (2748) than on the check. In the second experiment, oviposition on *P. trifoliata* accession 4172 was significantly lower compared with the check, but there were no significant differences in oviposition between the check and any of the other four *P. trifoliata* accessions (Table 5). Data variability precluded declaring that oviposition on CRC 3549 was reduced but this accession was one of the least colonized genotypes studied by Westbrook et al. (2011), and Richardson and Hall (2013) reported that not a single egg was laid on CRC 3549. Oviposition on the eight *P. trifoliata* accessions in the first two experiments was generally large relative to that reported by Richardson and Hall (2013). Of seven *P. trifoliata* accessions studied by Richardson and Hall (2013), only one accession appeared resistant in experiments 1 and 2 (CRC 3215) compared with six judged resistant by Richardson and Hall (2013). Because Richardson and Hall (2013) studied ACP oviposition on seedlings with both flush and mature leaves, we repeated experiments 1 and 2 but used seedlings with both types of leaves. In the third experiment (repeat of experiment 1 but with both young and mature leaves), significantly fewer eggs were observed on each of the three *P. trifoliata* accessions (1717, 3215 and 3572) compared with the check, with percentage differences ranging from 78 to 84% (Table 5). Based on the different outcomes of experiments 1 and 3 with respect to accessions 1717 and 3572, it can be hypothesized either that *P. trifoliata* seedlings with mature leaves are more resistant to oviposition or that 'Ridge Pineapple' susceptibility to oviposition is increased when mature leaves are present. In the fourth experiment (repeat of experiment 2 but with both young and mature leaves), significantly fewer eggs were laid on *P. trifoliata* accessions 4172 and 838 compared with numbers of eggs on 'Ridge Pineapple' (Table 5). However, the presence or absence of mature leaves on seedlings did not appear to make a difference in susceptibility to ACP oviposition on accessions 2552,

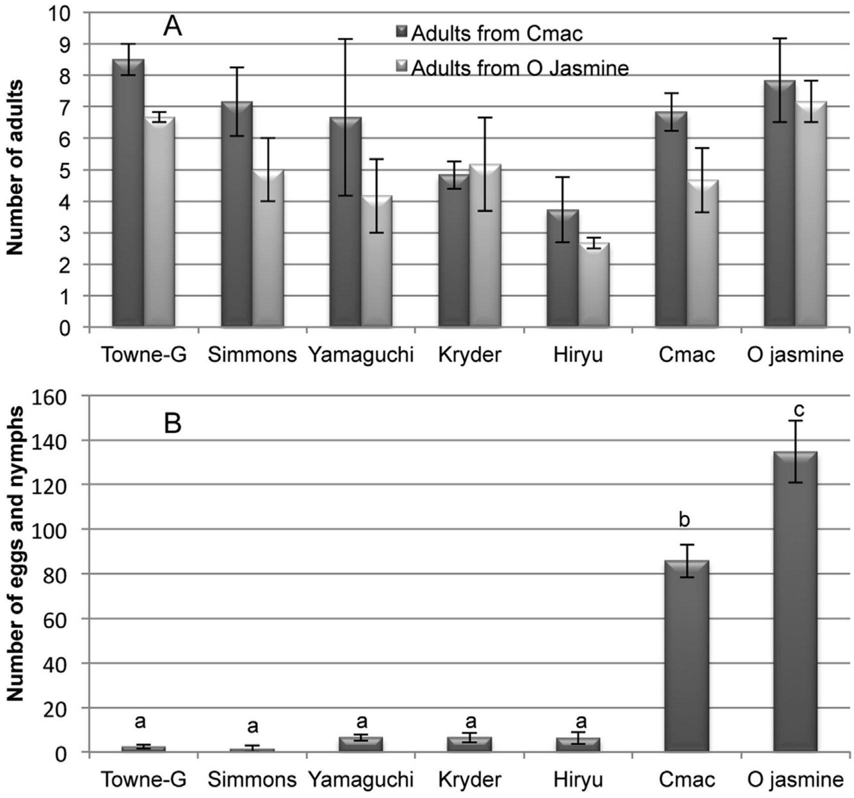

Fig. 1. **A**. Mean ± SEM number of adult *Diaphorina citri* remaining alive after 14 days of confinement to flush cuttings of *Citrus macrophylla*, orange jasmine, or one of five different *Poncirus trifoliata* accessions (starting number of adults was 25, n = 6). Three replications used adults from a colony on *C. macrophylla*, and the other 3 replications used adults acclimated to orange jasmine prior to the experiment –there were no significant differences in adult survival among the different plant species, but significantly greater numbers of adults survived when they were transferred from *C. macrophylla* than from orange jasmine. **B**. Mean ± SEM numbers of immature *D. citri* (eggs and nymphs) present on *P. trifoliata* flush cuttings after being infested for 14 days by adult *D. citri* (n = 6). Treatments having no letters in common are significantly different ($P = 0.05$), Tukey's HSD test.

3210 or 3549.

Richardson and Hall (2013) reported large reductions in oviposition on a majority of 47 accessions of *P. trifoliata* under no-choice conditions using seedlings with both flush and mature leaves, with many reductions in the range of 90–100% relative to oviposition on ACP-susceptible *C. macrophylla*. We judged only two of eight accessions to be resistant to oviposition in the absence of mature leaves while five of the eight were resistant when mature leaves were present compared with oviposition on ACP-susceptible *C. sinensis*. However, even in our experiments 3 and 4 where reductions in oviposition were relatively large on five accessions compared with *C. sinensis*, the reductions were smaller than anticipated based on data presented by Richardson and Hall (2013). A number of factors could be responsible, alone or in combination. Here, we used a different check genotype – *C. macrophylla* may stimulate more oviposition than *C. sinensis*, which could inflate differences in oviposition compared with oviposition on *P. trifoliata*. We used ACP from a colony maintained on orange jasmine while Richardson and Hall (2013) used ACP from a colony maintained on *C. macrophylla*. ACP may more readily oviposit on a host they were reared on. We conducted our experiments at 23 °C; Richardson and Hall (2013) conducted their tests at 27 °C. Other differences between our experiments and those by Richardson and Hall (2013) include the number of females studied (we studied 3 per plant, they studied 2) and the period during which females were allowed to oviposit (we allowed 2–4 days, they allowed 6). Perhaps the most significant difference was that we studied adults that were 15 d old while Richardson and Hall (2013) studied adults 6 d old. Oviposition by ACP older than 10 d has been reported to be significantly higher compared with younger ACP (Liu and Tsai, 2000). Extrapolation of data from Richardson and Hall (2013) indicated oviposition on their susceptible check ranged from 3.4 to 14.0 eggs per female per day, compared to 17.4 to 29.8 eggs per female per day in our experiments.

3.3. Oviposition behavior and survival on P. trifoliata flush cuttings

At the end of the 14-day period, there were no significant differences in survival of adult ACP among any of the *P. trifoliata* accessions nor between any of these accessions and either *C. macrophylla* or orange jasmine ($F_{6,\,28} = 1.99$; $P = 0.1379$) (Fig 1A). Over all treatments, a mean ± SEM ($n = 6$) of 48 ± 7% adults remained alive (treatment means ranged from 36 to 61%). Compared with the two susceptible control plants, significantly fewer numbers of immature ACP were observed on the *P. trifoliata* accessions ($F_{6,\,28} = 82.6$; $P < 0.0001$) (Fig. 1B). In fact, very few immatures were observed on the *P. trifoliata* plants compared to control plants. There was no significant interaction effect between the source of adult ACP (colony host plant) and numbers of immature ACP per flush cutting ($F_{6,\,28} = 1.2$; $P = 0.32$). The source of adult ACP (*C. macrophylla* or orange jasmine) had no significant effect on the number of immatures on flush ($F_{1,28} = 2.7$; $P = 0.11$), but there was a significant effect of source on ACP survival ($F_{1,\,28} = 8.12$; $P = 0.01$). Mean (±SEM, $n = 3$) number of adults remaining after 14 d was 5.1 ± 0.6 and 6.5 ± 0.6 on flush infested with adults from orange jasmine and *C. macrophylla*, respectively. Shifting ACP from *C. macrophylla* to orange jasmine to *P. trifoliata*, or from *C. macrophylla* to orange jasmine to *C. macrophylla*, might be more stressful to adult ACP than the single shift from *C. macrophylla* to *P. trifoliata*. Percent survival of adults from *C. macrophylla* was similar to that from orange jasmine when adults were transferred to orange jasmine (31 and 29%, respectively).

4. Summary

The results of the first study show that accessions of *P. trifoliata* exhibit resistance to ACP colonization under some conditions, but there are conditions under which *P. trifoliata* may be susceptible. In free-choice situations, traits associated with *P. trifoliata* that may reduce ACP attraction and oviposition could be masked by nearby susceptible plant genotypes. In each of the first study's four experiments, the plants were located in adjacent positions. Other factors could influence the level of resistance to oviposition including environmental conditions and plant age and physiology. With respect to ×*Citroncirus* accessions, there was no evidence in the first or the second study that traits responsible for reduced oviposition on *P. trifoliata* were passed to any of the hybrid accessions studied. Based on the results of the second study, in no-choice situations ACP laid fewer eggs on some but not all *P. trifoliata* accessions. Presence or absence of mature leaves may have influenced oviposition. In the third study, large reductions occurred in oviposition rates and subsequent nymph development on *P. trifoliata* flush cuttings in a no-choice situation. Over all three studies, a number of accessions of *P. trifoliata* were shown to exhibit resistance to ACP colonization. The following accessions were judged to be resistant: CRC 2554, 3206, 3207, 3209, 3218, 3338, 3412, 3486, 3547, 3548, 3588, and 3882. Research is needed to identify factors that influence *P. trifoliata* resistance to ACP oviposition. Reductions in oviposition on *P. trifoliata* could be a consequence of antixenosis whereby factors are missing that normally promote oviposition in susceptible plants, or there could be factors associated with *P. trifoliata* that deter oviposition. Reduced egg hatch and/or poor survival of nymphs on *P. trifoliata* could be a result of antibiosis.

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