Resistance of *Poncirus* and *Citrus* × *Poncirus* Germplasm to the Asian Citrus Psyllid

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ABSTRACT

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, has spread to citrus growing regions nearly worldwide and transmits phloem-limited bacteria (*Candidatus Liberibacter* spp.) that are putatively responsible for citrus greening disease. Host plant resistance may provide the most effective control, but ACP has a broad host range and resistance in *Citrus* spp. and relatives to ACP has not been widely documented. Very low abundances of ACP were found on two accessions of *Poncirus trifoliata* (L.) Raf. (hardy-orange) in a field survey. Therefore, we tested whether 81 accessions of *P. trifoliata* and ×*Citroncirus* spp. (hybrids of *P. trifoliata* and *Citrus* spp.) from the USDA-ARS National Clonal Germplasm Repository for Citrus and Dates were resistant to ACP by determining whether these accessions influence oviposition and lifespan of adults in no-choice tests. There was a higher abundance of eggs on the control (*Citrus macrophylla* Wester [alemow]) than nearly all accessions of *P. trifoliata*, and zero eggs were laid on 36% of the accessions. Additionally, more eggs were laid on the control than 10 of 34 accessions of ×*Citroncirus* spp. Lifespan of adults was approximately 2.5 to 5 times longer on 11 of the 17 trifoliates and trifoliate hybrids we tested. *Poncirus trifoliata* appears to have antixenosis and antibiosis resistance to ACP, but we must next identify the traits that promote resistance to create commercial varieties of citrus that reduce the population of ACP and lower the incidence of citrus greening disease.

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Abbreviations: ACP, Asian citrus psyllid; CRC, Citrus Research Center.

The Asian citrus psyllid (ACP), *Diaphorina citri*, is native to Asia but has spread to citrus growing regions nearly worldwide (Halbert and Manjunath, 2004; Halbert and Núñez, 2004; Pluke et al., 2005). In the United States, ACP has spread to Florida, Texas, California, Alabama, Arizona, Georgia, Louisiana, Mississippi, and South Carolina since the late 1990s and 2000s (Tsai and Liu, 2000; French et al., 2001; Bech 2009). Adult ACP feed on phloem in young stems and leaves of all ages but oviposit primarily on young elongating flush of cultivars of *Citrus* spp. (family Rutaceae) as well as other Rutaceae, including some grown as ornamental plants (Halbert and Manjunath, 2004; Westbrook et al., 2011). Nymphs are restricted to feeding on young stems and leaves and produce copious amounts of honeydew on which black sooty mold may develop, which blemishes foliage and fruit and reduces photosynthesis of leaves (Wang et al., 2001). Large infestations of ACP also cause direct damage to citrus by distorting or reducing the growth of flush (Michaud, 2004). However, most damaging to the tree is the fact that adult ACP transmit phloem-limited bacteria (*Candidatus Liberibacter* spp.) that are putatively responsible for citrus greening disease (huanglongbing). Citrus greening disease is considered the most serious disease of citrus worldwide because

Published in Crop Sci. 53:183–188 (2013).
doi: 10.2135/cropsci2012.02.0091

it causes trees to decline in productivity, causes misshapen sour-tasting fruit, and kills a tree within months to several years (McClean and Schwartz, 1970; Halbert and Manjunath, 2004; Bové, 2006).

Current management of ACP and citrus greening disease in the United States is largely by costly measures, such as intensive chemical control, removal of trees symptomatic for the disease, and planting disease-free nursery stock. Little is known about the extent to which classical biological control agents reduce populations of ACP. However, parasitoids have largely failed to establish or control ACP in the United States (Michaud, 2002, 2004; Hall et al., 2008) and control of ACP by parasitoids and predators may be incompatible with chemical control because natural enemies are often killed by chemicals (Smith and Peña, 2002; Hall and Nguyen, 2010; Hall and Richardson, 2012). Development of effective alternatives to insecticides and classical biological control for management of ACP is of critical importance. Host plant resistance, prevention of colonization or multiplication of ACP on a plant, ultimately may provide the most effective, economical, environmentally safe, and sustainable method of controlling ACP, especially if the plant also is resistant to other important pests of citrus. Traits that confer resistance to other species of insects have been documented among members of the orange subfamily Aurantioideae (Bowman et al., 2001; Luthria et al., 1989; Yang and Tang, 1988). Some *Citrus* spp. and relatives are apparently nonhosts or less preferred hosts to ACP (Halbert and Manjunath, 2004; Hall et al., 2010; Tsagkarakis and Rogers, 2010; Westbrook et al., 2011) and may influence the development, longevity, and reproduction of ACP (Tsai and Liu, 2000; Fung and Chen, 2006; Nava et al., 2007; Tsagkarakis and Rogers, 2010). For example, ACP laid fewer eggs, had lower survival, and developed slower on *Citrus reshni* hort. ex Tanaka (Cleopatra mandarin) (Tsagkarakis and Rogers, 2010). However, we found *C. reshni* to be a suitable host for oviposition (unpublished data, 2012), ACP is known to have a broad host range (Halbert and Manjunath, 2004; Westbrook et al., 2011), and resistance in *Citrus* spp. and related plant species to ACP has not been widely documented to date.

Eighty-seven accessions primarily in the Rutaceae, orange subfamily Aurantioideae, were surveyed in the field in Florida for abundance of ACP eggs, nymphs, and adults and very low abundances of all life stages of ACP were found on two accessions of *Poncirus trifoliata*, ‘Simmon’s trifoliate’ and ‘Little-leaf’ (Westbrook et al., 2011). *Poncirus trifoliata* is a trifoliate species that is graft compatible with *Citrus* spp., is used as rootstock in many citrus-growing regions (Krueger and Navarro, 2007; Ziegler and Wolfe, 1981), is an important parent in intergeneric hybrids with *Citrus* spp. (Krueger and Navarro, 2007), and may be resistant to another important pest of citrus, the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) (Richardson et al., 2011). Therefore, *P. trifoliata* may be useful in breeding programs as a potential source of genes that confer resistance to multiple species of insects. In this study, we test whether accessions of *P. trifoliata* (hereafter “trifoliates”) and ×*Citroncirus* (hereafter “trifoliate hybrids”), which are hybrids of *P. trifoliata* crossed with one or two *Citrus* spp. [i.e., *C. maxima* (Burm.) Merr. (pomelo), *C. paradisi* Macfad. (grapefruit), *C. sinensis* (L.) Osbeck, *C. reshni*, *C. aurantium* L. (sour orange), *Fortunella japonica* (Thunb.) Swingle (syn. *C. madurensis* Lour.) (kumquat), *C. reticulata* Blanco (mandarin orange), *C. limon* (L.) Burm. f. (lemon), and/or *C. tangelo* J. W. Ingram & H. E. Moore (tangelo)], are resistant to ACP by determining whether these accessions reduce oviposition and lifespan of adults.

MATERIALS AND METHODS

Plant Material

We obtained seeds from 86 accessions [each with a unique Citrus Research Center (CRC) number] of trifoliate and trifoliate hybrids from the USDA-ARS National Clonal Germplasm Repository for Citrus and Dates located at the University of California at Riverside (http://www.citrusvariety.ucr.edu/). Members of the Rutaceae vary greatly in their incidence of nucellar embryony (reviewed in Frost and Soost, 1968); therefore, some of the plants we tested were genetically identical to the female parent whereas others were sexual hybrids. We tried to select plants that were phenotypically uniform to increase the likelihood that they were genetically identical to the female parent, but we cannot rule out the possibility that sexually derived seedlings were included, which may differ in their susceptibility to ACP and increase variance in our results. We planted 25 seeds of each seed parent accession (hereafter “accession”) in individual plastic cells (3.8 by 21 cm) (SC-10 super cell Cone-tainers, Stuewe and Sons) containing sterile potting mix. Seedlings of 81 accessions (47 trifoliates and 34 trifoliate hybrids) were successfully propagated in a greenhouse.

Experiment 1: Oviposition

After growing plants for 4 to 5 mo in a greenhouse, the 81 accessions were separated into smaller groups of 3 to 11 and screened for resistance to oviposition over time (4 May to 24 June 2011) due to logistical necessity. We clipped approximately 2.5 cm of the central stems of the plants within a group 1 wk before each test date to induce flush. On each test date we chose 10 plants of each accession that had young, unexpanded flush and placed onto each plant two females and one male ACP from a laboratory colony at the United States Horticultural Laboratory in Fort Pierce, FL (described by Hall et al., 2007). On each test date we also put ACP on 10 plants of *Citrus macrophylla*, which is highly susceptible to colonization by ACP (Westbrook et al., 2011) and served as the control. The control plants were roughly the same age as the test plants and also were clipped to induce flush. We chose ACP that had been adults for approximately 6 d to ensure they were reproductive (Wenninger and Hall, 2007). We enclosed plants with plastic cylinders (37 by 255 mm) that had four circular side windows (25 mm) and a top window covered with white silk fabric. The open bottom of each cylinder was pressed into the Cone-tainers to prevent ACP from escaping. The plants were arranged in a random complete block design in a tray and placed in a growth chamber set at 27°C, 48% relative humidity, and 14 h daily illumination. The number of eggs on each plant was counted 6 d after inoculation under a dissection microscope.

Experiment 2: Adult Lifespan

We placed one adult female ACP each on eight seedlings of each trifoliate hybrid (n = 10) that was resistant to oviposition, five accessions of trifoliate hybrids that were susceptible to oviposition (‘Rangpur × Troyer’, ‘Sanford’, ‘African shaddock × Rubidoux’, ‘Citrangor’, and ‘C-190’), two accessions of *P. trifoliata* that were resistant to oviposition (‘Towne ‘G’’ and ‘Flying Dragon B’), and the susceptible control (*C. macrophylla*) (see Results). Using accessions that varied in susceptibility to oviposition allowed us to test whether there was a correlation between this trait and adult lifespan. Asian citrus psyllids were chosen that had been adults for less than 24 h to standardize their age and ensure that they had not mated (Wenninger and Hall, 2007). We enclosed plants with plastic cylinders and placed them in a random complete block design in trays in a growth chamber at the settings previously described. Each adult ACP was checked daily until their death to determine whether lifespan differed on seedlings of these parent accessions.

Statistical Analyses

Differences among seedlings of the 81 accessions and the control in abundance of eggs were tested by negative binomial models (PROC GENMOD [SAS Institute, 2002]). Abundance of eggs on seedlings of accessions was compared to the control within a date but not across dates because oviposition by ACP on the control accession varied over time. The LSMEANS statement was then used to estimate separation between pairs of means (SAS Institute, 2002; Sokal and Rohlf, 1995). We also used negative binomial models to test for differences in the lifespan of adult ACP among the 18 accessions in Exp. 2 and to test whether adult lifespan was correlated with the relative susceptibility of accessions to oviposition. Sample size was reduced for some accessions in both experiments because of insufficient numbers of plants that germinated or produced flush.

RESULTS AND DISCUSSION

The abundance of eggs differed among progeny of the 81 accessions (Table 1). There was a higher abundance of eggs on the control (*C. macrophylla*) than on 42 of the 46 accessions of trifoliates, and 36% of the accessions averaged zero eggs. The only accessions that did not have fewer eggs than the control were ‘Towne ‘F’’, ‘Rich 7-5’, ‘Rich 22-2’, and ‘Little-leaf’. Each of these accessions averaged fewer than 26 eggs compared to over 100 on the control, but high variance of eggs laid on the control on the two dates these accessions were sampled prevented statistically significant results. Given the low number of eggs laid on these accessions and the apparent resistance of the other 42 accessions of trifoliate, we consider it unlikely that these four accessions are highly susceptible. More eggs also were laid on the control than 10 of 34 accessions of trifoliate hybrids (Tables 1 and 2). Four accessions each of trifoliates and trifoliate hybrids that we present in this paper also were also tested in China for susceptibility to oviposition and yielded similar results: trifoliates were resistant to oviposition and trifoliate hybrids varied in their susceptibility (D.G. Hall and R. Chuanqing, personal communication, 2012).

Table 1. Mean (±SEM) number of eggs laid by *Diaphorina citri* on 81 accessions of *Poncirus trifoliata* and ×*Citroncirus* spp. compared to a susceptible host plant (*Citrus macrophylla*). Botanical names, Citrus Research Center (CRC) number, and plant categories (except “control”) are derived from the Citrus Variety Collection of the University of California at Riverside. Trifoliate hybrids are identified as a citrange (*P. trifoliata* × *Citrus sinensis*), citrumelo (*P. trifoliata* × *Citrus paradisi*), or hybrid (*P. trifoliata* × other *Citrus* spp.).

Date	Negative binomial model[†]	Botanical name of seed parent (CRC no.)	Plant category	n	Mean no. eggs (SEM)
4 May	$\chi^2 = 32.8$, Num df = 6, Den df = 63, $P < 0.001$	*C. macrophylla* (3842)	Control	10	102.8 (48)a[‡]
		×*Citroncirus* sp. ‘C-35’ (3912)	Citrange	10	127.8 (59)a
		×*Citroncirus* sp. ‘C-32’ (3911)	Citrange	10	122.7 (57)a
		×*Citroncirus* sp. ‘Morton’ (1463)	Citrange	10	91.2 (42)ab
		P. trifoliata ‘$^{\#}$27’ (3938)	Trifoliate	10	29.0 (14)b
		P. trifoliata ‘Kryder 55-1’ (3486)	Trifoliate	10	4.1 (2.0)c
		P. trifoliata ‘Benoit’ (3547)	Trifoliate	10	3.7 (1.8)c
10 May	$\chi^2 = 30.9$, Num df = 6, Den df = 62, $P < 0.001$	*C. macrophylla* (3842)	Control	10	105.4 (38)a
		×*Citroncirus* sp. ‘Citremon’ (1449)	Hybrid	10	150.4 (23)a
		×*Citroncirus* sp. ‘Benton’ (3908)	Citrange	10	147.7 (53)a
		×*Citroncirus* sp. ‘S-302 Citranguma’ (3415)	Hybrid	9	134.7 (51)a
		×*Citroncirus* sp. (3336)	Citrange	10	119.3 (43)a
		×*Citroncirus* sp. ‘Cunningham’ (271)	Citrange	10	64.0 (23)a
		P. trifoliata ‘Rich 16-6’ (3485)	Trifoliate	10	4.0 (1.6)b
12 May	$\chi^2 = 23.4$, Num df = 5, Den df = 54, $P < 0.001$	*C. macrophylla* (3842)	Control	10	108.9 (67)a
		×*Citroncirus* sp. ‘Glen Citrangedin’ (3573)	Hybrid	10	159.6 (99)a
		P. trifoliata ‘Towne F’ (3572)	Trifoliate	10	22.0 (14)ab
		P. trifoliata ‘Kryder 16-6’ (3210)	Trifoliate	10	12.5 (7.8)b
		P. trifoliata ‘Kryder Medium’ (3212)	Trifoliate	10	5.4 (3.4)bc
		P. trifoliata ‘Kryder 55-5’ (3215)	Trifoliate	10	1.9 (1.3)c

Table 1. Continued.

Date	Negative binomial model[†]	Botanical name of seed parent (CRC no.)	Plant category	*n*	Mean no. eggs (SEM)
18 May	$\chi^2 = 15.6$, Num df = 6, Den df = 63, $P = 0.016$	*C. macrophylla* (3842)	Control	10	168.4 (141)a
		×*Citroncirus* sp. ‘C-190’ (3889)	Citrumelo	10	70.9 (60)ab
		P. trifoliata ‘Webber-Fawcett $^{\#}$22’ (2552)	Trifoliate	10	12.7 (11)bc
		P. trifoliata ‘Taylor’ (3571)	Trifoliate	10	10.7 (9.1)bc
		P. trifoliata ‘Marks’ (3588)	Trifoliate	10	6.0 (5.1)c
		P. trifoliata ‘$^{\#}$26” (3939)	Trifoliate	10	4.9 (4.2)c
		P. trifoliata ‘Kryder 28-3’ (3219)	Trifoliate	10	3.9 (3.3)c
19 May	$\chi^2 = 95.8$, Num df = 5, Den df = 54, $P < 0.001$	*C. macrophylla* (3842)	Control	10	85.8 (36)a
		×*Citroncirus* sp. ‘Citrumelo’ (1452)	Citrumelo	10	133.8 (56)a
		×*Citroncirus* sp. ‘Trifeola’ (3954)	Hybrid	10	94.5 (40)a
		P. trifoliata ‘Kryder 8-5’ (3218)	Trifoliate	10	2.5 (1.2)b
		P. trifoliata ‘Swingle nucellar’ (4138)	Trifoliate	10	0.0 (0.0)c
		P. trifoliata (4009)	Trifoliate	10	0.0 (0.0)c
23 May	$\chi^2 = 42.1$, Num df = 6, Den df = 61, $P < 0.001$	*C. macrophylla* (3842)	Control	10	108.0 (59)a
		×*Citroncirus* sp. (3821)	Hybrid	10	84.6 (46)a
		×*Citroncirus* sp. ‘X639’ (3957)	Hybrid	10	62.4 (34)a
		×*Citroncirus* sp. ‘Rangpur X Troyer’ (3997)	Hybrid	10	39.1 (21)a
		×*Citroncirus* sp. ‘Citrumelo’ (3348)	Citrumelo	8	7.6 (4.7)b
		P. trifoliata ‘Kryder 5-5’ (3586)	Trifoliate	10	0.2 (0.2)c
		P. trifoliata ‘Big-leaf’ (4006)	Trifoliate	10	0.2 (0.2)c
26 May	$\chi^2 = 54.2$, Num df = 5, Den df = 54, $P < 0.001$	*C. macrophylla* (3842)	Control	10	84.7 (69)a
		×*Citroncirus* sp. ‘Morton’ (1463)	Citrange	10	13.8 (11)b
		×*Citroncirus* sp. ‘Citrandarin’ (2618)	Hybrid	10	11.0 (9.0)b
		×*Citroncirus* sp. ‘Savage’ (275)	Citrange	10	8.1 (6.7)b
		P. trifoliata ‘English’ (3548)	Trifoliate	10	0.0 (0.0)c
		P. trifoliata ‘Simmons’ (3549)	Trifoliate	10	0.0 (0.0)c
31 May	$\chi^2 = 12.2$, Num df = 6, Den df = 61, $P = 0.058$	*C. macrophylla* (3842)	Control	10	107.3 (65)a
		×*Citroncirus* sp. ‘Uvalde’ (2865)	Citrange	9	118.6 (75)a
		P. trifoliata ‘Rich 22-2’ (3211)	Trifoliate	10	26.0 (16)ab
		×*Citroncirus* sp. ‘Citrangor’ (1447)	Hybrid	9	25.1 (16)ab
		P. trifoliata ‘Little-leaf’ (4008)	Trifoliate	10	23.8 (14)ab
		P. trifoliata ‘Rich 7-5’ (3587)	Trifoliate	10	22.3 (14)ab
		×*Citroncirus* sp. ‘Citremon’ (1448)	Hybrid	10	9.9 (6.0)b
1 June	$\chi^2 = 75.7$, Num df = 6, Den df = 57, $P < 0.001$	*C. macrophylla* (3842)	Control	10	111.6 (81)a
		×*Citroncirus* sp. ‘Sanford’ (276)	Citrange	7	42.0 (37)ab
		×*Citroncirus* sp. ‘Swingle’ (3771)	Citrumelo	7	31.7 (28)bc
		P. trifoliata ‘Little-leaf’ (4007)	Trifoliate	10	10.0 (7.4)c
		P. trifoliata ‘Small leaf’ (4017)	Trifoliate	10	1.4 (1.1)d
		P. trifoliata ‘Flying dragon’ (3330B)	Trifoliate	10	0.5 (0.4)d
		P. trifoliata ‘Florida’ (2862)	Trifoliate	10	0.0 (0.0)d
7 June	$\chi^2 = 58.0$, Num df = 3, Den df = 28, $P < 0.001$	*C. macrophylla* (3842)	Control	8	56.9 (22)a
		×*Citroncirus* sp. ‘S-281 Citrangelo’ (3552)	Hybrid	4	38.0 (21)a
		P. trifoliata ‘Kryder 60-2’ (3213)	Trifoliate	10	0.3 (0.2)b
		P. trifoliata ‘Rich 12-2’ (3209)	Trifoliate	10	0.0 (0.0)b
9 June	$\chi^2 = 83.7$, Num df = 7, Den df = 61, $P < 0.001$	*C. macrophylla* (3842)	Control	10	71.0 (48)a
		×*Citroncirus* sp. ‘Yuma’ (3205)	Citrange	7	11.3 (9.2)b
		×*Citroncirus* sp. (3881)	Citrange	8	10.8 (8.2)b
		P. trifoliata ‘Frost’ (3484)	Trifoliate	5	2.0 (2.0)c
		P. trifoliata ‘USDA’ (1498)	Trifoliate	10	1.5 (1.1)c
		P. trifoliata ‘Benecke’ (3338)	Trifoliate	10	1.5 (1.1)c
		P. trifoliata ‘Texas’ (2861)	Trifoliate	9	0.0 (0.0)c
		P. trifoliata ‘Kryder 15-3’ (3217)	Trifoliate	10	0.0 (0.0)c
15 June	$\chi^2 = 34.4$, Num df = 4, Den df = 33, $P < 0.001$	*C. macrophylla* (3842)	Control	8	40.8 (31)a
		×*Citroncirus* sp. ‘Sacaton’ (3414)	Citrumelo	5	30.2 (29)a
		P. trifoliata ‘Barnes’ (2554)	Trifoliate	7	3.3 (2.7)b
		×*Citroncirus* sp. ‘Troyer’ (1459)	Citrange	10	2.9 (2.0)b
		P. trifoliata (3888)	Trifoliate	8	0.0 (0.0)b

Table 1. Continued.

Date	Negative binomial model[†]	Botanical name of seed parent (CRC no.)	Plant category	*n*	Mean no. eggs (SEM)
16 June	χ^2 = 48.6, Num df = 6, Den df = 57, $P < 0.001$	*C. macrophylla* (3842)	Control	10	43.6 (37)a
		×*Citroncirus* sp. 'Carrizo' (2863)	Citrange	10	46.8 (40)a
		×*Citroncirus* sp. 'Rusk' (301)	Citrange	4	12.0 (16)ab
		P. trifoliata 'Towne G' (3207)	Trifoliate	10	10.8 (0.7)b
		P. trifoliata 'Argentina' (3206)	Trifoliate	10	3.8 (1.2)b
		P. trifoliata 'Australian' (3151)	Trifoliate	10	0.0 (0.0)c
		P. trifoliata 'Hiryu' (3882)	Trifoliate	10	0.0 (0.0)c
24 June	χ^2 = 122, Num df = 10, Den df = 83, $P < 0.001$	*C. macrophylla* (3842)	Control	10	141.8 (97)a
		×*Citroncirus* sp. 'Citradia' (1436)	Hybrid	6	83.5 (74)ab
		×*Citroncirus* sp. 'African shaddock × Rubidoux trifoliate' (3969)	Citrumelo	10	59.7 (41)abc
		P. trifoliata 'English dwarf' (3976)	Trifoliate	6	30.2 (27)bc
		×*Citroncirus* sp. 'Citradia' (1438)	Hybrid	6	19.8 (18)cd
		P. trifoliata 'Rubidoux' (838)	Trifoliate	10	7.2 (5.0)d
		P. trifoliata 'Yamaguchi' (3412)	Trifoliate	10	0.2 (0.2)e
		P. trifoliata 'Pomeroy' (1717)	Trifoliate	10	0.1 (0.1)e
		P. trifoliata 'Flying dragon' (3330A)	Trifoliate	10	0.0 (0.0)e
		P. trifoliata 'Jacobson' (3411)	Trifoliate	6	0.0 (0.0)e
		P. trifoliata 'Large flower' (NA)	Trifoliate	10	0.0 (0.0)e

[†] Num df, numerator degrees of freedom; Den df, denominator degrees of freedom.

[‡] Means with different letters are significantly different (means separation test, $P < 0.05$).

Lifespan of adults was approximately 2 to 5 times longer on *C. macrophylla* than on one of the two trifoliates ('Flying Dragon B') and 11 of the 15 trifoliate hybrids (χ^2 = 40.3, df = 17,125, $P = 0.001$; Table 2). Three accessions we tested for adult lifespan were tested in China ('Flying Dragon', unnamed CRC 3881, and 'Troyer'), and lifespan was reduced on all three in China (D.G. Hall and R. Chuanqing, personal communication, 2012), whereas 'Troyer' seemed to be relatively suitable for adults in the United States. Although some accessions that were resistant or susceptible to oviposition retained the same relative susceptibility to adult ACP, lifespan of adults on average was not different between accessions that were susceptible (mean ± SEM = 40.7 ± 5.4 d) or resistant (35.6 ± 3.4 d) to oviposition (χ^2 = 0.67, df = 1, 141, $P = 0.41$), indicating that accessions that are resistant to one life stage may not be resistant to other life stages. Citrus greening disease is transmitted by nymphs and adults of ACP, and adults may survive long enough to transmit the disease even on the accessions that are most resistant to adult ACP. Therefore, it is necessary to test whether ACP survive long enough on these accessions to transmit the disease and whether these accessions are resistant to the disease, which appears to be the case with at least one plant species related to citrus, *Murraya paniculata* (L.) Jack (orange jasmine) (Walter et al., 2012).

We must identify genotypic and phenotypic plant traits that confer resistance to ACP. Resistance to ACP is likely conferred by chemical mechanisms and not structural characteristics of the plant, because ACP will settle to feed on trifoliates in no-choice tests and their stylets insert into phloem cells as they do in susceptible plants (unpublished data, 2012). *Poncirus trifoliata* seems to have antixenosis-type

Table 2. Mean (±SEM) lifespan of adult *Diaphorina citri* on 17 accessions of *Poncirus trifoliata* and ×*Citroncirus* spp. that differentially influenced oviposition by *D. citri*. Trifoliate hybrids are identified as a citrange (*P. trifoliata* × *Citrus sinensis*), citrumelo (*P. trifoliata* × *Citrus paradisi*), or hybrid (*P. trifoliata* × other *Citrus* spp.).

Botanical name of seed parent (CRC[†] no.)	Plant type (based on susceptibility to oviposition)	Mean (SEM) lifespan of adult psyllids (d)
C. macrophylla (3842)	Susceptible control	90.0 (26)a[‡]
P. trifoliata 'Towne G' (3207)	Resistant trifoliate	71.8 (21)ab
×*Citroncirus* sp. 'Rangpur × Troyer' (3997)	Susceptible hybrid	50.3 (14)ab
×*Citroncirus* sp. 'Citrandarin' (2618)	Resistant hybrid	46.5 (13)abc
×*Citroncirus* sp. 'Troyer' (1459)	Resistant citrange	46.3 (13)abc
×*Citroncirus* sp. 'Swingle' (3771)	Resistant citrumelo	44.6 (14)abcd
×*Citroncirus* sp. 'Yuma' (3205)	Resistant citrange	41.3 (12)abcd
×*Citroncirus* sp. 'Citremon' (1448)	Resistant hybrid	35.9 (10)bcde
×*Citroncirus* sp. 'Sanford' (276)	Susceptible citrange	34.8 (10)bcde
×*Citroncirus* sp. 'Morton' (1463)	Resistant citrange	29.9 (8.7)cde
×*Citroncirus* sp. 'African shaddock × Rubidoux trifoliate' (3969)	Susceptible citrumelo	28.4 (8.3)cde
×*Citroncirus* sp. 'Savage' (275)	Resistant citrange	27.4 (8.0)cde
×*Citroncirus* sp. 'Citrumelo' (3348)	Resistant citrumelo	25.0 (7.3)cde
P. trifoliata 'Flying Dragon B' (3330B)	Resistant control	22.5 (6.6)cde
×*Citroncirus* sp. 'Citrangor' (1447)	Susceptible hybrid	22.4 (6.6)cde
×*Citroncirus* sp. 'Citradia' (1438)	Resistant hybrid	20.1 (5.9)de
×*Citroncirus* sp. 'C-190' (3889)	Susceptible citrumelo	17.8 (5.3)e
×*Citroncirus* sp. (3881)	Resistant citrange	17.8 (5.3)e

[†] CRC, Citrus Research Center.

[‡] Means with different letters are significantly different (means separation test, $P < 0.05$

resistance because ACP do not heavily colonize this species in field surveys (Westbrook et al., 2011) or lay eggs on it, so perhaps a plant volatile repels ACP or a plant volatile necessary for attraction is lacking. *Poncirus trifoliata* also may have antibiosis-type resistance as indicated by reduced lifespan of adults on some trifoliates and trifoliate hybrids. Resistance of trifoliate hybrids to ACP is likely inherited from *P. trifoliata* and not the *Citrus* sp. with which it is crossed because *Citrus* spp. are usually highly susceptible to ACP (Halbert and Manjunath, 2004; Westbrook et al., 2011). A number of advanced selections of citrus with *P. trifoliata* in their pedigree are available in citrus breeding programs, so we are screening these selections for resistance to ACP in the field and greenhouse because some of them are close to commercial quality. Another approach to produce commercial varieties of citrus that are resistant to ACP is to identify genes that confer resistance and transfer them to commercial varieties via transgenic or intragenic methods (Rommens et al., 2007). Ultimately, our results demonstrate that resistance of citrus to ACP could play an important role in the management of citrus greening disease.

Acknowledgments

We thank M. Watson and J. Malicoate for assistance in the laboratory, two anonymous reviewers for improving the content of this manuscript, and the Florida Citrus Research and Development Foundation for financial support.

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