Survey of *Poncirus trifoliata* hybrids for resistance to colonization by Asian citrus psyllid

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Asiatic huanglongbing, also known as citrus greening or yellow shoot disease, is a serious citrus disease presumably caused by a bacterium ('*Candidatus* Liberibacter asiaticus'; Rhizobiaceae) transmitted by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Bové 2006). Some *Citrus* species such as certain mandarins (*Citrus reticulata* Blanco; Rutaceae) exhibit various degrees of tolerance to the pathogen, but all *Citrus* (Rutaceae) species are susceptible to infection and some of the most economically important types of *Citrus* can quickly succumb to the disease, notably sweet oranges (*Citrus sinensis* [L.] Osbeck; Rutaceae) and grapefruit (*Citrus paradisi* Macfad.; Rutaceae). *Citrus* species highly susceptible to the disease decline in health and productivity to the point they lose their economic value. There is no cure for infection, and management programs aimed at the psyllid have ultimately been ineffective in preventing the introduction and spread of the pathogen in North America (Hall et al. 2013a, b).

Poncirus trifoliata (L.) Raf. (Rutaceae) is a close relative of and readily hybridizes with sweet oranges, grapefruit, and other *Citrus* species. Although genetically similar to *Citrus*, *P. trifoliata* is a deciduous relative that produces inedible fruit. The proven value of *P. trifoliata* in citrus production is its performance as a rootstock, increasing resistance to soilborne pests, and conferring cold protection along with other desirable traits to the aboveground scion-portion of a tree. Citrus breeders have developed hybrids of *P. trifoliata* and *Citrus* spp. that are more broadly adapted than *P. trifoliata*, and are the primary rootstocks used in US citriculture. Two traits of *P. trifoliata* are of interest in the pursuit of solutions to huanglongbing. One is tolerance to the pathogen, which has been noted in both pure *P. trifoliata* and some of its hybrids (Albrecht & Bowman 2012). The other trait of interest in pure *P. trifoliata* is resistance to colonization by the psyllid (Westbrook et al. 2011; Hall et al. 2015). *Diaphorina citri* sometimes oviposits on *P. trifoliata*, but lays significantly fewer eggs than on *Citrus* species, which are highly susceptible to oviposition (Richardson & Hall 2013; Hall et al. 2017). Efforts continue to identify the trait (s) responsible for reduced oviposition on *P. trifoliata*, because these might be transferred to *Citrus* as a management tactic against *D. citri*. With this in mind, Hall et al. (2017) conducted a field survey to assess oviposition rates of *D. citri* on 4 citrange cultivars (hybrids between *P. trifoliata* and sweet orange). There was no evidence from this survey that traits conferring reduced oviposition on *P. trifoliata* were passed to these hybrids. However, many *Poncirus* hybrids have been and continue to be generated by breeders, and one cannot conclude from 4 hybrids alone that traits discouraging oviposition have not been passed to or expressed by other hybrids.

Presented here are the results of 3 field surveys of *Poncirus* hybrids looking for resistance to colonization by *D. citri*. The surveys were conducted in different plantings of trees located at a USDA grove near Fort Pierce, Florida, USA (27.4356833°N, 80.4309194°W). The plantings were subjected to routine fertilization and irrigation, herbicides, copper treatments for plant diseases, and occasional spray oil treatments, but no hard insecticides. The hybrids surveyed included citranges; citrandarins (hybrids with mandarin orange, *C. reticulata*); citrumelos (hybrids with grapefruit, *C. paradisi*); citradias (hybrids with sour orange, *Citrus aurantium* L.; Rutaceae); citrangumas (hybrids between citrange and satsuma mandarin, *Citrus unshiu* Marcow.; Rutaceae); citremons (hybrids with lemon, *Citrus limon* [L.] Osbeck; Rutaceae); and hybrids between pomelo, *Citrus maxima* (Burm.) Merr. (Rutaceae), and *Poncirus*. The trees were periodically visited to collect 2 flush shoots appropriate for oviposition, although sometimes only 1 shoot could be found, and occasionally no flush was present on a tree. The shoots were transported to a laboratory and examined under a microscope to count eggs and nymphs, the latter of which were counted separately by instar. Combined egg + nymph counts per sample (converted to logs) and percentages of samples infested by fifth instar nymphs (arcsine transformed) were subjected to analyses of variance (ANOVA) (PROC GLM) (SAS Institute 2010), and results were reported with untransformed means.

In a 2017 survey, 27 genotypes were monitored for eggs and nymphs, including 20 new citranges from USDA and University of Florida breeders, 1 citrandarin, 3 complex hybrids, 2 *Citrus* species as susceptible checks, and 1 pure *Poncirus* resistant check. Five trees of each were sampled monthly from May to Sep. The 6-yr-old trees were in a large replicated planting (previously described by Hall et al. 2017) with conventional citrus cultivars, pure trifoliate cultivars, and many experimental hybrids. Significantly fewer immatures were observed on the pure *Poncirus* cultivar than on any other cultivar ($F_{25, 474}$ = 5.9; P = < 0.0001) (Table 1). There were no significant differences between *Citrus* and any *Poncirus* hybrid with respect to infestation levels of immatures, but oviposition was somewhat reduced on 'Ftp646130,' the only hybrid tested with *P. trifoliata* in the pedigree of both parents. There was no obvious deterrence of nymphal development on any of the hybrids based on the frequency of fifth instar nymphs observed, and no significant differences ($F_{25, 406}$ = 0.9; P = 0.58) were found among the cultivars with respect to percentages of samples with these nymphs.

In the second survey, oviposition rates were investigated on 6 different types of *Poncirus* hybrids. The seed source of the trees was the Citrus Research Center, Riverside, California, USA, and the specific CRC accession number was known for each cultivar. The trees were 5 yr old, and there

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Table 1. Mean number of immature Asian citrus psyllid per flush shoot in a 6-yr-old field planting of *Citrus*, *Poncirus trifoliata*, and their hybrids (survey 1). All hybrids except 'Ftp646130' had 1 parent that was either pure *Poncirus* or a *Poncirus* hybrid; both parents of 'Ftp646130' were *Poncirus* hybrids.[a]

Genotype group	Cultivar	Mean number immatures per flush shoot	Mean percentage of infested samples with fifth instar nymphs
Citrus	'Hamlin' *C. sinensis*	40.9 ab	61.1 a
Citrus (Tangor)	'Temple'	40.7 ab	65.0 a
Citrandarin	'US-897'	26.0 ab	52.4 a
Citrange	'B11R1T22'	55.7 ab	47.8 a
Citrange	'B11R1T34'	47.1 ab	31.3 a
Citrange	'B11R5T57'	35.3 ab	54.6 a
Citrange	'B6R18T3'	35.8 ab	57.9 a
Citrange	'B6R18T6'	38.6 ab	40.0 a
Citrange	'B6R18T7'	56.4 ab	54.2 a
Citrange	'FD-X-SHAM-#1'	48.8 ab	50.0 a
Citrange	'FD-X-SHAM-#4'	70.7 a	60.0 a
Citrange	'FD-X-SUCC-#15'	29.9 ab	45.8 a
Citrange	'FD-X-SUCC-#19'	24.5 ab	33.3 a
Citrange	'FD-X-SUCC-#5'	25.5 ab	60.0 a
Citrange	'SFB16R1T55'	45.5 ab	27.3 a
Citrange	'SFB16R2T54'	47.0 ab	25.0 a
Citrange	'SFB6R1T53'	48.4 ab	38.1 a
Citrange	'SFB7R1T44'	66.2 ab	57.1 a
Citrange	'SFB7R1T56'	45.5 ab	41.2 a
Citrange	'SFB7R1T58'	52.4 ab	61.1 a
Citrange	'SFB7R2T47'	42.3 ab	44.4 a
Citrange	'SFB7R2T59'	34.4 ab	42.9 a
Citrange	'SFB7R2T89'	33.7 ab	34.8 a
Complex citrumelo	'US-119'	35.9 ab	41.7 a
Complex citrumelo	'2610'	21.7 ab	60.9 a
Complex citrumelo	'Ftp646130'[b]	15.7 b	41.2 a
Poncirus	'Rubidoux'	6.9 c	37.5 a

[a]Means in the same column followed by the same letter are not significantly different ($P > 0.05$), Ryan-Einot-Gabriel-Welsch multiple range test.
[b]Hybrid between '2610' and ([pummelo × *Poncirus*] × 'Succory' sweet orange).

were 4 trees of each cultivar (only 3 trees of 2 'Sacaton' accessions) in a replicated 0.25 ha planting of many selections along 3 rows (175 m long, 0.8 m tree spacing). Two pure *Poncirus* cultivars were sampled as resistant checks. No pure *Citrus* accessions were included in the planting, thus the citrange 'Uvalde' was surveyed as a susceptible check (Hall et al. 2017). The trees were sampled 9 times from Feb to Sep 2018. Infestation densities were relatively low throughout the survey, often with low percentages of flush shoots infested. Therefore, analyses were conducted on the maximum number of immature psyllids per flush shoot per tree. Significant differences among cultivars were found in infestation levels of *D. citri* ($F_{14, 110} = 6.1$; $P = < 0.0001$), but there was no evidence of resistance to *D. citri* oviposition in any of the hybrids compared to the check (Table 2). Fifth instar nymphs were observed on all hybrids except 'Sacaton 2,' indicating that at least some nymphs fully developed on most hybrids. The data were too variable to declare any significant differences among cultivars with respect to percentages of samples with fifth instar nymphs ($F_{14, 169} = 0.2$; $P = 0.99$).

In the third survey, oviposition rates were assessed on a single tree of each of 6 different groups including 6 *Poncirus* hybrids. Two species of *Severinia* (Mantidae) (a close relative of *Citrus*) were included for comparison purposes. The 19-yr-old trees were scattered across 7 rows (140 m long, 4.6 m tree spacing) in a 0.7 ha planting of diverse germplasm maintained for breeding purposes. The trees were sampled 9 times from Mar to Sep 2018. As in the second survey, analyses were conducted on the maximum number of immature psyllids per flush shoot per tree. Significant differences were found among cultivars with respect to colonization by *D. citri* ($F_{14, 102} = 13.2$; $P = < 0.0001$). There was no evidence of reduced oviposition on the hybrids 'Cpb-40197,' 'Savage,' or 'Willits' (Table 2). Oviposition was reduced on 3 citrumelo hybrids ('Cpb-4481,' 'Sacaton,' and 'Yuma'; the latter was once considered a citrange but recent investigations indicate this is a citrumelo [M. Roose, unpublished]), but factors unrelated to resistance to oviposition may have been involved. This caution is provided primarily because only 1 tree of each cultivar was available for the survey. While in the second survey colonization on 3 of 6 citrumelo hybrids was not significantly different on 'Flying Dragon,' all 6 of these citrumelos, including 2 'Sacaton' cultivars, were as susceptible to colonization as a susceptible check. Tree age was 1 notable difference between the citrumelo cultivars in the 2 surveys. Fifth instar nymphs were frequently observed on 3 hybrids (1 citrandarin and 2 citranges). The 2 *Severinia* species were as susceptible to colonization as the *Citrus* hybrid accessions, although no fifth instars were observed during the limited observations of these 2 trees. Data on percentages of samples with fifth instars were too variable to declare any significant differences among cultivars ($F_{12, 40} = 0.8$; $P = 0.61$).

The results of this study indicated that simple hybrids between *Poncirus* and *Citrus* species lack the marked resistance pure *Poncirus* exhibits to colonization by *D. citri*. This was true even during late spring and early summer when larger populations of *D. citri* are common in Florida citrus. Based on 'Ftp646130,' further investigations are warranted on the resistance of hybrids when both parents have *Poncirus* in their pedigree.

Summary

Genetic traits in *Poncirus trifoliata* (Rutaceae) are known to confer resistance to colonization by the Asian citrus psyllid, but field surveys indicated these traits were not expressed in any of 24 simple hybrids between *P. trifoliata* and sweet orange. No resistance to colonization by the psyllid was observed in 14 other types of *P. trifoliata* hybrids. Three citrumelo hybrids appeared to be resistant in 1 survey, but in another survey, none of

Table 2. Maximum numbers of immature Asian citrus psyllid per flush shoot observed in 5-yr-old (survey 2) and 9-yr-old (survey 3) field plantings of *Poncirus trifoliata* hybrids. All hybrids had 1 parent that was either pure *Poncirus* or a *Poncirus* hybrid.[a]

Genotype group	Genotype or cultivar	CRC[b]	Maximum number immatures per sample	% samples with fifth instar nymphs
5-yr-old trees				
Citrange	'Uvalde' (susceptible check)	2865	30.4 a	36.4 a
Citrange	'Carrizo'	2863	44.3 a	30.8 a
Complex citrange	'Citrangor'	1447	43.5 a	31.3 a
Citradia	*Poncirus* × *C. aurantium*	1436	32.4 a	35.0 a
Citranguma	'S-302'	3415	49.9 a	30.0 a
Citremon	*Poncirus* × *Citrus limon*	1449	52.6 a	30.0 a
Pomelo hybrids	African shaddock × *Poncirus*	3969	28.3 a	27.8 a
Citrumelo	'C-190'	3889	49.4 a	33.3 a
Citrumelo	*C. paradisi* × *Poncirus*	3821	41.1 a	47.6 a
Citrumelo	*Poncirus* × *C. paradisi*	1452	23.1 ab	28.6 a
Citrumelo	'Sacaton 1'	3337	17.7 ab	23.1 a
Citrumelo	'Sacaton 2'	3414	29.2 ab	0.0 a
Complex citrumelo	'US-119'	3998	20.2 a	26.3 a
Poncirus	'Rubidoux'	838	2.2 c	33.3 a
Poncirus	'Flying Dragon'	3330	9.1 bc	33.3 a
19-yr-old trees				
Citrus	*Citrus macrophylla* Wester	—	14.9 abc	42.9 a
Citrus	'Jackson' *C. paradisi*	—	35.8 ab	50.0 a
Citrus	'Ridge Pineapple' *C. sinensis*	—	53.0 ab	40.0 a
Citrus	'Triumph' *C. paradisi*	—	33.0 ab	42.9 a
Citrus	'Volkamer' *C. limon*	—	21.6 ab	33.3 a
Citrandarin	'Cpb-40197'	—	35.8 ab	16.7 a
Citrange	'Savage'	—	27.4 ab	83.3 a
Citrange	'Willits'	—	68.3 a	62.5 a
Citrumelo	'Yuma'	—	3.3 cde	0.0 a
Citrumelo	'Sacaton'	—	1.6 de	0.0 a
Complex citrumelo	'cpb-4481'[c]	—	3.2 cde	50.0 a
Citrus relative	*Severinia buxifolia* (Poir.) Ten.	—	15.1 bcd	0.0 a
Citrus relative	*Severinia disticha* (Blanco) Swingle	—	20.4 ab	0.0 a
Poncirus	'Yamaguchi'	—	0.0 e	—
Poncirus	'Rubidoux'	—	0.0 e	—

[a]Means in the same column followed by the same letter are not significantly different ($P > 0.05$), Ryan-Einot-Gabriel-Welsch multiple range test.
[b]Citrus Research Center accession number (http://citrusvariety.ucr.edu/citrus/index.html)
[c](*Citrus paradisi* × *Poncirus*) × open pollination

6 citrumelos exhibited resistance. Interestingly, colonization was somewhat reduced on a hybrid with *P. trifoliata* in the pedigree of both parents, suggesting the possibility that traits suppressing oviposition may be recovered through similar crosses.

Key Words: citrus greening; huanglongbing; *Diaphorina citri*; Liberibacter

Sumario

Se sabe que los rasgos genéticos en *Poncirus trifoliata* (Rutaceae) confieren resistencia a la colonización por parte del psílido asiático de los cítricos, pero los sondeos de campo indicaron que estos rasgos no se expresaron en ninguno de los 24 híbridos simples entre *P. trifoliata* y naranja dulce. No se observó resistencia a la colonización por el psílido en otros 14 tipos de híbridos de *P. trifoliata*. Tres híbridos de citrumelo parecieron ser resistentes en 1 sondeo, pero en otro sondeo, ninguno de los 6 citrumelos mostró resistencia. Curiosamente, la colonización se redujo algo en un híbrido con *P. trifoliata* en el pedigrí de ambos parientes, lo que sugiere la posibilidad de que los rasgos que suprimen la oviposición puedan recuperarse a través de cruces similares.

Palabras Clave: cítricos enverdecimiento; huanglongbing; *Diaphorina citri*; Liberibacter

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