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ORIGINAL CONTRIBUTION

Suitability of native North American Rutaceae to serve as host plants for the Asian citrus psyllid (Hemiptera: Liviidae)

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Diaphorina citri, host acceptance, host plant, native Rutaceae, North America

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Abstract

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, a vector of citrus huanglongbing, is now present in all citrus-producing states in the USA and Mexico. In addition to citrus, the insect can reproduce on several other plant species in the *Rutaceae* family; orange jasmine (*Murraya* spp.) and curry leaf (*Bergera koenigii*) are among its preferred hosts. There are several indigenous Rutaceae species in North America, and some are popular ornamentals. A study was therefore initiated to determine the suitability of some of these plants for feeding and development of the psyllid in choice and no-choice experiments. *D. citri* was found to reproduce successfully on *Choisya ternata*, *C. arizonica* and *Helietta parvifolia* in no-choice tests, but preferentially selected orange jasmine and curry leaf for feeding and reproduction, in choice tests. On *Amyris madrensis*, *A. texana* and *Zanthoxylum fagara*, adult psyllids laid eggs which hatched, but no successful nymphal development was recorded beyond the first instars. No oviposition was recorded on *Esenbeckia berlandieri*, *Ptelea trifoliata* and *Casimiroa tetrameria*, although adult psyllids were able to survive on these species for several days. Results showed that *C. ternata*, *C. arizonica* and *H. parvifolia* can serve host plants of *D. citri* and this constitutes the first report of these plants serving as host for *D. citri*. The findings of the present study suggest that native rutaceous host plants can serve as host plants and thus affect *D. citri* population dynamics and the epidemiology of Huanglongbing, the deadly citrus greening disease whose pathogen is vectored by *D. citri*. Thus, area-wide management of this pest also should target these riparian habitats where these host plants are present with *D. citri* biological control agents for sustainable management of this pest.

Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is the vector of citrus Huanglongbing (HLB) caused by *Candidatus* Liberibacter asiaticus (CLas), now considered to be the most serious diseases of citrus worldwide (Bové 2006). The insect has spread from its Asian origin to other citrus growing areas; its arrival in Florida was recorded in 1998 (Knapp et al. 1998) and was discovered soon afterwards in Texas (French et al. 2001) and in Mexico (Lopez-Arroyo et al. 2005). It subsequently appeared in California and Arizona (Gomes 2008, 2009).

The detection of HLB in Florida in 2005 (Halbert 2005) elevated concerns about the potential threat this vector–pathogen combination posed to the citrus industry of the United States and triggered off major research activities. To date, HLB has been detected in all major citrus-producing states in the United States, including Florida and Texas where the disease has spread significantly in commercial groves and residential citrus, and California where only a few isolated trees have been detected HLB positive in residential areas. In Florida where the disease has been present for a decade, its incidence is nearly 100%. From 2006 to 2012, it has caused an economic impact of some

$4.5 billion with a concomitant loss of over 8000 jobs (Hodges and Spreen 2012; Spreen et al. 2014). In Texas, HLB was first detected in 2012 and has since been confirmed in more than 100 groves and 170 residential sites (da Graça et al. 2015).

Strategies to reduce the incidence of HLB have mainly concentrated on the production of clean nursery plants in psyllid-resistant structures and management of the psyllid and pathogen in commercial citrus groves. However, several other plants within the Aurantioideae and especially within the Rutaceae are known hosts of *D. citri* (Halbert and Manjunath 2004; Thomas 2011). In an extensive review, Halbert and Manjunath (2004) listed 23 non-*Citrus* hosts, all in the family Rutaceae. Two non-*Citrus* host species are considered preferred hosts of *D. citri* (Subandiyah et al. 2008; Westbrook et al. 2011). In Florida, observations were carried out on species of *Zanthoxylum*; no *D. citri* were ever found on *Z. clava-hercules*, but were occasionally present on *Z. fagara* and *Z. ailanthoides* (Halbert and Manjunath 2004; Westbrook et al. 2011). Although adult *D. citri* readily survived on four species of *Zanthoxylum* (*Z. clava-herculis* L., *Z. coriaceum* A. Rich., *Z. flavum* Vahl. and *Z. fagara* (L.) Sarg.), no reproduction was recorded on these native Rutaceae in Florida (Russell et al. 2014). The white sapote *Casimiroa edulis* growing in Florida was not colonized as a host by *D. citri* (Halbert and Manjunath 2004; Westbrook et al. 2011). However, the role of these native and cultivated rutaceous host plants in the dynamics of *D. citri* populations and spread of CLas are largely unknown.

In South Texas, Everitt et al. (2002) listed the following rutaceaous species as native: Mountain torchwood (*Amyris madrensis*), Texas torchwood (*A. texana*), Jopoy (*Esenbeckia berlandieri*), Baretta (*Helietta parvifolia*) and Prickly ash species (*Zanthoxylum clava-herculis*, *Z. fagara* and *Z. hirsutum*). Fucik (1978) also reported *A. madrensis, Z. fagara, H. parvifolia* and the wafer ash or hop tree (*Ptelea trifoliata*) as suitable native rutaceous plants used for ornamental landscaping in the Lower Rio Grande Valley where citrus is cultivated. Other native Rutaceae which occur in West Texas and Mexico include the Mexican orange (*Choisya ternata* and *C. dumosa*) (Miller 2013) and Dutchman's breeches (*Thamnosma texana*) (Rickett 1970). *Choisya ternata* been cultivated for medicinal purposes in Mexico for many years and is grown in Europe as an ornamental plant (Creche et al. 1993).

This study was conducted to determine whether indigenous North American Rutaceae could serve as host plants for *D. citri*, and the role they could play in the population dynamics of this pest in Texas. Choice and non-choice studies, in which settling and reproduction by *D. citri* were scored, were conducted to evaluate the acceptability of selected native rutaceous plants to the psyllid. The results of this study will be used to evaluate the need to include these plants in area-wide management plans aimed at protecting the citrus industry.

Materials and Methods

Host plants

In South Texas, orange jasmine, *Murraya exotica* (L.), is a commonly grown ornamental plant as ornamental is residential area, and, along with *Bergera koenigii* (L.), Sprengel are among the most favoured hosts for the psyllid (Subandiyah et al. 2008). These two known *D. citri* host plants were therefore used as positive controls in these experiments. A survey of ornamental and citrus nurseries was initially conducted to acquire any native rutaceous plant available. Potted plants of various ages of nine native rutaceous species were purchased from local nurseries and an out-of-state nursery (Table S1). These plants were selected for testing based on their availability. Plants were transferred to 3.7-l black plastic pots containing Metro-Mix® 360 potting soil (www.sungro.com) and maintained on elevated benches in a greenhouse under ambient temperature and no supplemental lighting. Plants were watered as needed but fertilized twice a month with a solution of dilute fertilizer (N : P : K 20 : 20 : 20) *ad libitum*. All plants were at least 12 months old when used in bioassays.

Diaphorina citri source

Diaphorina citri source used in the experiments were obtained from a laboratory-reared colony maintained at the Citrus Center, Texas A&M University-Kingsville in Weslaco, TX, for a minimum of 3 years. The colony was initially established from adults collected from a mature grapefruit grove (*Citrus x paradisi* cv. Rio Red) and maintained on orange jasmine plants for several generations. These individuals were reared at 24 ± 2°C, 65 ± 5% RH and light regime of 14 : 10 (L : D) hr in the laboratory. New plants with profuse flush shoots were frequently placed in the rearing cages to provide suitable plants for oviposition and feeding in the colony. Only newly emerged and mated adults (ca. 3 day-old) are used in the bioassays.

Adult *D. citri* survivorship in no-choice tests

To perform non-choice experiments, one plant of each of the 11 plant species (nine native rutaceous plants plus orange jasmine and curry leaf plant as positive controls) was placed in a 50 × 50 × 90 cm rearing cage. Climatic conditions of 21–28°C and 40–70% relative humidity were prevailing in the greenhouse with ambient lighting during the experiments. Newly emerged and mated adult psyllids were individually collected from the source colonies using an aspirator. The psyllids were sexed by examining their abdomen using a standard stereo microscope. A total of 20 psyllids (10 ♂♂ and 10 ♀♀) were transferred onto the plant in the cage in each cage. Plants and cages were inspected daily to record the number of surviving psyllid adults for two weeks to prevent any confounding effect with possible emerging adult progeny. Three cages were used per species for evaluating *D. citri* survivorship on the different test plants. The homogeneity of *D. citri* survivorship curves on the different plant species was tested using a likelihood ratio test with the Proc Lifetest of SAS. Bonferroni-corrected pairwise technique suggested by McDonald and Gardner (2000) was applied for comparing any pair of plants using the likelihood ratio test. As there were 11 plants tested, 55 pairwise comparisons were made; hence, for any comparison to be significant at the $P < 0.05$ level, the P-value of the likelihood ratio test had to be <0.00091 ($=0.05/55$).

Diaphorina citri development bioassays in no-choice tests

The same experimental set-up was used to determine the plant suitability for *D. citri* reproduction. All plants were pruned, irrigated and fertilized as previously described to promote profuse flush shoot production. Plants with approximately 8–12 young expanding flush shoots were selected and individually placed inside experimental cages. Ten pairs of newly emerged and mated *D. citri* were released in each cage. Five days after release, all psyllid adults were removed and the plants were inspected for the presence for *D. citri* oviposition. Two flush shoots were randomly selected per plant and using a 5× hand magnifier glass, the numbers of eggs present on each of these flush shoots per plant species were counted *in situ*. After counting the number of eggs, all plants were continuously maintained in the cages and inspected daily for the numbers nymphs and adults. Nymphal emergence was recorded by scoring their presence or absence of flush shoots for five consecutive days after counting the eggs. No attempt was made to count individual numbers of nymphs on flush shoots. The time to each adult emergence was recorded. Emerging adults were removed from the cages, sexed and counted. Five replications were set-up per plant species. Plants that allowed successful development of *D. citri* were classified as host plants for the insect.

Plants and flush shoots were categorized as infested or non-infested based on the presence of eggs. Any plant with at least one infested flush shoot was classified as infested. Due to low frequencies of certain categories (<5), Fisher's exact multinomial test of independence was used to compare the ratio of infested plants and flush shoots between plant species (Zar 1999). When there was evidence of significance differences between plant species, a post hoc exact binomial test was used to compare any two plant species by adjusting the P-value with the Bonferroni-corrected pairwise technique (McDonald and Gardner 2000).

The numbers of eggs laid on each plant, the duration of immature development and the total numbers of adult progeny produced per plant were subjected to one way analysis of variance to test whether plant species affected *D. citri* oviposition behaviour and development. When significant F-values ($P < 0.05$) were obtained, mean numbers of eggs on plant were discriminated using the Student–Newman–Keuls (SNK) test (Zar 1999).

To determine the overall effect of plant species on *D. citri* development, the growth index (GI) defined as the ratio between the total numbers of adult progeny emerging from each plant species (i.e. ultimate survivorship) and the mean immature developmental time (herein recorded as the time from the beginning of the experiment to emergence of adult) was calculated (Sétamou et al. 1999). GI values are positive with zero indicating that the plant is not suitable for *D. citri* development. A log-likelihood ratio test (*G*-test) of goodness-of-fit was used to compare GI values obtained from the different plants.

Multichoice bioassays

Three native host plants (*C. ternata*, *C. arizonica* and *H. bareta*) and the two known host plants (*B. koenigii* and *M. exotica*) allowed successful development of *D. citri* with at least one adult progeny emerging from cages in non-choice bioassays. A multichoice bioassay was then conducted to evaluate the preference of these five plants species for *D. citri* settling and feeding in the laboratory. Young flush shoots of similar sizes were collected from these plants and simultaneously provided to adult psyllids under continuous lighting (~3000 lux illumination) of for five consecutive days. These flush shoots (~ 10 cm long) were individually inserted in aquatubes containing hydroponic solution (General Hydroponics, Sebastopol, CA, USA) soon after collection to maintain their turgidity. These aquatubes were placed equidistantly on a perforated circular plate placed inside a clear glass desiccator (Secador® Techni-Dome® 360 Vacuum Desiccator, Bel-Art Products, www.belart.com). Hydroponic solution was added to the aquatubes as needed. Twenty pairs of young mated adult *D. citri* (20 ♂♂ and 20 ♀♀) were released inside the desiccator by placing the tube containing these insects at the centre of the perforated plate and allowing them to freely select their feeding and oviposition sites. The numbers of psyllids recorded feeding on flush shoots were recorded daily for the five consecutive days. Following the five days observation period, *D. citri* adults were removed and the number of eggs on each flush shoot was counted. This multichoice experiment was repeated five times with flush shoot position rotated for each replication.

A *G*-test of independence was first used to compare the number of *D. citri* on flush shoots of the different host plants. Because of the constant shift observed in *D. citri* adult location on flush shoots in the desiccator, a closed testing procedure with bootstrap resampling using the Proc Multtest of SAS was then applied to the number of adult psyllids to determine their host plant preference (Westfall et al. 2011). The relative settling preference of *D. citri* adults on these five host plants was calculated as a deviation of proportion of adults settled on each plant shoot from the overall mean of psyllids that settled. A *G*-test was used to compare the proportion of flush shoots infested with *D. citri* eggs in these choice tests.

Statistical analyses

All statistical analyses were performed using the SAS for windows version 9.4 (SAS Institute Inc. 2011). Numbers of *D. citri* eggs and adults were log(x+1)-transformed before analysis, however, non-transformed means are presented.

Results

Effect of plant species on adult *D. citri* survivorship in no-choice tests

The lifetest analysis showed that adult *D. citri* survivorship significantly varied with the plant species ($\chi^2 = 490.81$; d.f. = 10; $P < 0.0001$) (fig. 1). Comparisons of various subsets of plant species indicated that *A. texana*, *C. tetrameria E. berlandieri* and *P. trifoliata* were the least suitable for *D. citri* survivorship. No surviving *D. citri* was recorded on these plants after 10 d. Survivorship was significantly higher on *A. madrensis*, *C. ternata*, *H. parvifolia* and *Z. fagara* relative to the four least suitable plants; however, very few (5%) to no psyllids survived after 14 d. *D. citri* survivorship was highest on *M. exotica*, *B. koenigii* and *C. arizonica* on which >68% of adult psyllids were alive at the 14 d when they were censored. Comparative analysis of the survivorship curves between these three plants indicated that they were equally suitable for *D. citri* survival ($\chi^2 = 1.02$; d.f. = 2; $P = 0.60$).

Suitability of rutaceous plants for *D. citri* development

Diaphorina citri laid eggs on flush shoots of all but one of the 11 plant species tested in no-choice tests (table 1). *E. berlandieri* was not suitable for *D. citri* oviposition despite the survival of adult psyllids for up to 10 days on this plant (fig. 1). This may probably be due to the architecture of the young shoot that develops as open individual leaves in contrast to other species that form leaf clusters upon emergence. *D. citri* lays eggs in the leaf folds and of its host plants (Tsai and Liu 2000). Fisher's exact test for plant and flush shoot infestation with eggs yielded P-values <0.0001, suggesting that there was evidence that *D. citri* exhib-

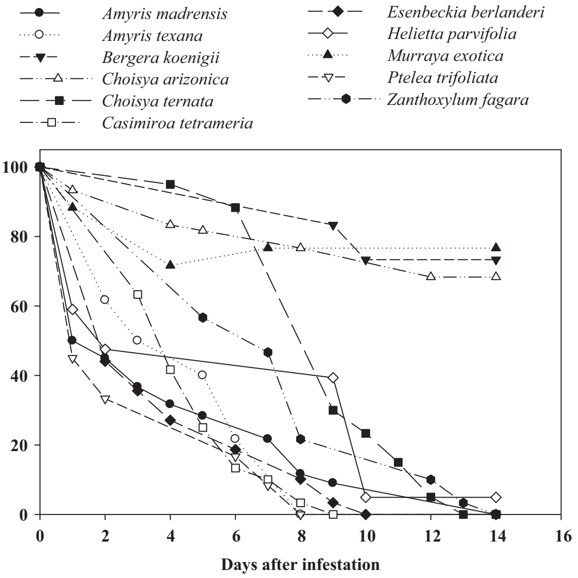

Fig. 1 Survivorship of adult *Diaphorina citri* on various rutaceous plants over two weeks in rearing cages.

ited significant oviposition preference for flush shoots of certain plant species (fig. 2). Adult psyllid laid eggs on all individual tested plants of the two known host plants *B. koenigii* and *M. exotica* as well as on all the three native rutaceous plants, *C. arizonica*, *C. ternata* and *Z. fagara*. All flush shoots of these five plant species were also infested with *D. citri* eggs (fig. 2). *D. citri* less frequently deposited eggs on flush shoots of *C. tetrameria, A. texana* and *P. trifoliata*. The numbers of eggs recorded per flush shoot were significantly dependent on the plant species (F = 33.48; d.f. = 10, 99; P < 0.0001). Significantly, more eggs were laid on *B. koenigii*, *C. arizonica*, *C. ternata* and *M. exotica* than any other host plants (table 1).

No *D. citri* nymphs emerged from eggs laid on flush shoots of *A. texana* and *C. tetrameriana* (table 1), suggesting that egg did not successfully complete their development on these plants. Psyllid nymphs were observed on *A. madrensis*, *P. trifoliata* and *Z. fagara,* but no adult emerged from these plants. In contrast, *D. citri* adult progeny emerged from the two known host plants (*B. keonigii* and *M. exotica*) and three native rutaceous plants (*C. arizonica*, *C. ternata* and *H. parvifolia*) suggesting their suitability for *D. citri* immature development and reproduction. However, the total number of adult progeny produced per cage significantly varied with the plant species (F = 21.66; d.f. = 10, 35; P < 0.0001). Significantly higher numbers of adults emerged from *B. koenigii*, *M. exotica* and the two *Choisya* species as compared to *H. parvifolia* (table 1).

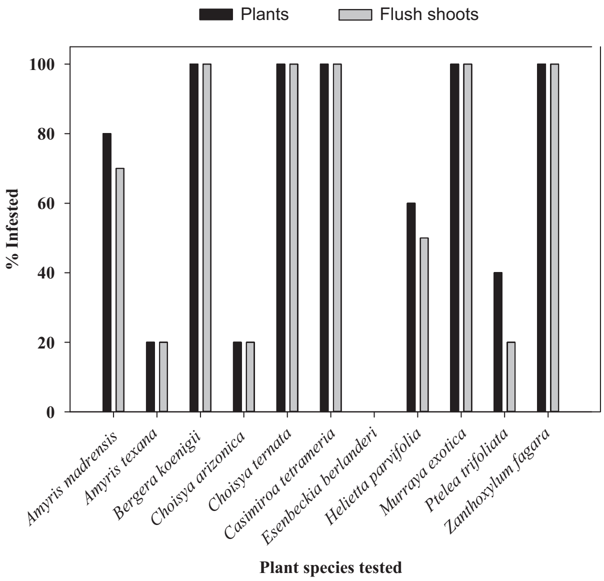

Fig. 2 Percent plants and flush shoots infested with *Diaphorina citri* eggs in no-choice tests of plant acceptability for oviposition [Fisher exact tests resulted in P < 0.0001 for both plants (n = 8 55) and flush shoots (n = 110)].

The duration of *D. citri* immature development was also host plant dependent (F = 7.90; d.f. = 4, 89; P < 0.0001) with significantly slower development on

Table 1 *Diaphorina citri* reproduction parameters on selected rutaceous plants in no-choice experiments

Plant species	Number of eggs laid per flush	Nymphal developmental time (days)	Total adult progeny produced per plant	Growth Index (GI)
Amyris madrensis	8.5 ± 3.8 cd	-[†]	0 d	-[‡]
Amyris texana	3.3 ± 2.7 d	-[*]	0 d	-[‡]
Bergera koenigii	85.1 ± 12.6 a	15.86 ± 1.58 bc	561 ± 210 a	35.3
Casimiroa tetrameria	2.5 ± 1.3 d	-[*]	0 d	-[‡]
Choisya arizonica	54.6 ± 17.6 ab	28.75 ± 1.70 a	125 ± 24 b	4.36
Choisya ternata	41.2 ± 13.5 b	16.35 ± 0.58 b	117 ± 10 b	6.14
Esenbeckia berlanderi	0 ± 0 e	-	0 d	-[‡]
Helietta parvifolia	11.6 ± 3.7 c	22.64 ± 1.6 ab	43 ± 23 c	1.9
Murraya exotica	52.9 ± 17.2 ab	13.34 ± 0.55 c	231 ± 48 ab	17.3
Ptelea trifoliata	2.3 ± 1.8 d	-[†]	0 d	-[‡]
Zanthoxylum fagara	18.4 ± 7.3 c	-[†]	0 d	-[‡]
Statistic	F = 33.48	F = 7.90	F = 21.66	*G* = 56.65
d.f.	10, 99	4, 89	10, 35	4
P-value	<0.0001	<0.0001	<0.0001	<0.0001

Average values expressed as means ± SEM. Data were log(x + 1)-transformed before analysis, but back transformed means are presented. Means followed by the same letter are not different at P < 0.05 using the Student–Newman–Keuls test.

[*] No nymphs emerged.

[†] Nymphs developed beyond first instars but no adult emergence occurred.

[‡] Data could not be calculated.

C. arizonica. The fastest development of *D. citri* immature was recorded on the two known host plants (*B. koenigii* and *M. exotica*) and the native Rutaceae *C. ternata* (table 1).

The performance of *D. citri* on the five plant species on which successful development occurred was evaluated by calculating the growth index (GI) as defined by Sétamou et al. (1999). GI values on the different host plants were examined via the chi-squared test for goodness-of-fit, and results showed a significant effect of plant species on *D. citri* performance ($G = 56.65$; d.f. = 4; $P < 0.0001$; table 1). The performance of *D. citri* was highest when reared on *B. koenigii* followed by *M. exotica*, while the psyllid was least successful on the three native rutaceous host plants. The positive relationship between the mean number of eggs laid per flush (x) and the growth index on a host plant (y) ($y = -2.78 + 0.34x$; d.f. = 9; $R^2 = 0.77$; $P = 0.0004$) suggested that *D. citri* growth potential on its host plants could be explained by its oviposition potential.

Multichoice bioassays

Multichoice experiments were conducted with the five host plants that allowed successful development of psyllid in the present study. *D. citri* adults exhibited strong host selection preference in their settling behaviour in multichoice tests ($G = 223.31$; d.f. = 4; $P < 0.0001$) (table 2). Analysis of contrasts showed that more *D. citri* settled on the known host plants *B. koenigii* and *M. exotica* than on the native host plants (*C. arizonica*, *C. ternata* and *H. parvifolia*). About 69% of all *D. citri* adults were recorded on flush shoots of the two known host plants in these choice tests, whereas the proportions of *D. citri* that selected flush shoots of each of the native host plants were significantly <20% (table 2). Comparison of adult settling preference showed that when tested simultaneously, *D. citri* exhibited strong preference for *B. koenigii* and *M. exotica* as indicated by the positive relative deviations compared to the three native host plants that had negative values from the assumption of equality of preference of adult psyllids for all plants (fig. 3).

Table 2 *Diaphorina citri* settling and oviposition preferences in multichoice tests (n = 25 for each host plant)

Plant species	Number of adults settling on flush shoots	% Adult settling per host plant	% Flush shoots with *D. citri* eggs
Bergera koenigii	8.8 ± 0.85 a*	41.3 a*	68 a*
Choisya arizonica	3.4 ± 0.46 c	16.1 c	52 b
Choisya ternata	1.5 ± 0.33 d	6.9 d	28 c
Helietta parvifolia	1.7 ± 0.34 d	8.1 d	0 d
Murraya exotica	5.9 ± 0.75 b	27.6 b	76 a
Statistic	F = 27.73	*G* = 223.13	*G* = 38.95
d.f.	4,120	4	4
P-value	<0.0001	<0.0001	<0.0001

Average values expressed as means ± SEM of all 25 flush shoots per host plant.

*Means followed by the same letter are not different at P < 0.05 using the adjusted P-value with the stepdown bootstrap method.

Examination of flush shoot choice for oviposition indicated a significant effect of host plant species on *D. citri* egg laying behaviour ($G = 38.95$; d.f. = 4; $P < 0.0001$). Similar to the pattern displayed for adult settling behaviour, *D. citri* preferentially selected *B. koenigii* and *M. exotica* for oviposition with, respectively, 68% and 76% of their flush shoots infested with eggs. *D. citri* laid eggs on about half of *C. arizonica* flush shoots; whereas only 28% of *C. ternata* flush shoots were selected for oviposition. Although *D. citri* successfully completed the development on *H. parvifolia* in non-choice tests, no oviposition was recorded on its flush shoots in choice tests consistent with the observed lower adult settling (8.1%).

Discussion

Confining adult *D. citri* in rearing cages with rutaceous plants producing flush shoots in non-choice studies clearly indicated that five of the test plants were suitable for psyllid reproduction. As expected,

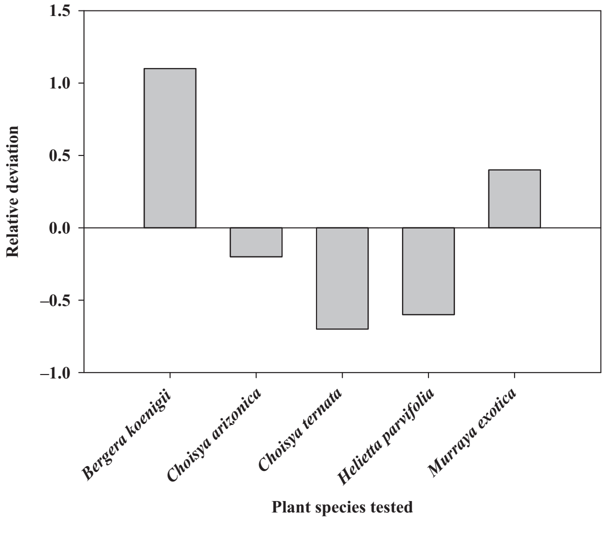

Fig. 3 Settling preference of *Diaphorina citri* adults on five host plants as indicated by the deviation of relative proportion from the hypothesis of equality.

D. citri successfully reproduced on its preferred host plants, *B. koenigii* and *M. exotica*; successful reproduction was also observed on three native Texas species, *C. arizonica*, *C. ternata* and *H. parvifolia*. These findings constitute the first report of these three native species as host plants for *D. citri*.

As shown by the higher oviposition rate, faster immature development and the higher number of adult progeny, psyllid performance on *B. koenigii* and *M. exotica* exceeded that of the three native species. Consequently, the growth index which is an indication of the effect of food quality on both survival rate and developmental time of insects (Sétamou et al. 1999) was highest on *B. koenigii* and *M. exotica* (table 2). These two host plants were also preferentially selected for settling and oviposition in choice tests. The observation of higher *D. citri* preference for and performance on *B. koenigii* and *M. exotica* over the native Rutaceae is not surprising as these known host plants are also of Asian origin and may have co-evolved with the psyllid (Halbert and Manjunath 2004; Hall et al. 2012). Both are classified as members of the Aurantioideae subfamily along with *Citrus*, while *Zanthoxylum, Choisya* and *Esenbeckia* are in the Rutoideae subfamily, and *Amyris, Casimiroa, Helietta* and *Ptelea* are in the Toddalioidea subfamily (Swingle 1967).

Diaphorina. citri adults used in the present study were obtained from a colony maintained for several generations on *M. exotica*, but the higher performance and settling preference of the psyllid on *B. koenigii* in no-choice and multichoice bioassays, respectively, indicated that the natal host plant did not significantly affect *D. citri* host selection behaviour. Thus, in the present study, *D. citri* adults did not exhibit enhanced preference for *M. exotica* on which its nymphs completed development as suggested by the Hopkins' host selection principle (Hopkins 1917). However, it is worth noting that flush shoots of *B. koenigii* and *M. exotica* were equally infested with eggs in multichoice tests, despite the higher settling preference exhibited by *D. citri* adults for the former host plant. Settling of *D. citri* adults on flush shoots of its host plants may be for various reasons including feeding, resting, courtship and mating, or oviposition purposes, while selection of flush shoots for oviposition is a critical decision that affects the survival and fitness of the progeny. *D. citri* is a very mobile insect (Boina et al. 2009; Sétamou et al. 2014), and settling on plants can constantly change. In contrast, oviposition is a final step of a *D. citri* decision-making process to ensure fitness of its progeny. *D. citri* host selection for feeding and oviposition can be dependent on its host plant volatile profiles (Wenninger et al. 2009; Patt and Sétamou 2010; Martini et al. 2014), but subtle differences in reflected light of their flush shoots might also be important in host colonization. The preference of *B. koenigii* and *M. exotica* over native Rutacae plants in choice tests suggests that these known host plants emit volatile organic compounds that are highly attractive to adult *D. citri* as compared to the three newly identified Texas native host plants. Similar to flush shoot selection for settling, *D. citri* preferentially laid eggs on flush shoots of *B. koenigii* and *M. exotica* as compared to *H. parvifolia*, *C. arizonica* and *C. ternata* in choice bioassays. Surprisingly, no eggs were laid on *H. parvifolia* in these choice tests, despite being suitable for *D. citri* reproduction in no-choice tests, but 52% and 28% of *C. arizonica* and *C. ternata* flush shoots were, respectively, colonized with *D. citri* eggs.

In no-choice assays, *D. citri* laid eggs on flush shoots of plants that did not support immature development, but significantly fewer eggs were deposited on these non-host plants as compared to those suitable for nymphal development with the exception of *Z. fagara*. The number of eggs deposited on flush shoots of *Z. fagara* was comparable to that of *H. parvifolia* on which *D. citri* successfully reproduced. Among the non-suitable plants for *D. citri* development, eggs did not hatch on flush shoots of two species (*A. texana* and *C. tetrameria*), while eggs hatched but nymphs did not successful developed on *P. trifoliata, A. madrensis* and *Z. fagara*. The non-emergence of adult progeny on *P. trifoliata, A. madrensis* and *Z. fagara* was due to high nymphal mortality. Poor nutritional quality of their phloem sap and/or accessibility of phloem sap by developing nymphs could be the main factors explaining such high mortality rates on these plants. Examination of all Rutaceae used in the present study indicated that all non-suitable plants are characterized by three-parted leaves and a few of these plants have hairy young shoots, suggesting that morphological characteristics of plants may also be important in *D. citri* reproduction. In a few instances, dried and shrivelled *D. citri* eggs were observed on flush shoots of *C. tetrameria*. On *Z. fagara*, although nymphal development occurred to late instars, none of these nymphs successfully developed to adult stage, suggesting this plant was not suitable for *D. citri* reproduction confirming observations made by Russell et al. (2014). Another related species, *Z. ailanthoides* was reported as an occasional host of *D. citri* (Halbert and Manjunath 2004; Westbrook et al. 2011). The high nymphal and adult mortality (fig. 2) observed on flush shoots of *P. trifoliata, A. madrensis* and *Z. fagara* suggest strong antibiosis of these plants towards

D. citri, and this could open avenues into evaluating compounds or nutrients present in phloem sap that could interfere with *D. citri* development.

The reasons for *D. citri* oviposition on plants that do not support complete nymphal development are not fully understood. Many ecological factors including differential host plant suitability among life stages or simply a mistake on the mother's part leading to oviposition on 'sink plants' (Mayhew 2001; Clark et al. 2012) could explain these observations. Nonetheless, higher numbers of eggs were recorded on flush shoots of suitable host plants in the present study. The positive relationship between the mean numbers of eggs laid on flush shoots and the growth index, as defined by Sétamou et al. (1999), adds support to the preference/performance-type hypothesis stating that herbivorous insects show colonization preference for host plants that will maximize their fitness (Craig et al. 1989; Gripenberg et al. 2010; Clark et al. 2012).

Because of the use of *H. parvifolia*, *C. arizonica* and *C. ternata* as landscape ornamentals in Texas and many parts of Mexico, their confirmation as *D. citri* host plants bears a significant importance in their role in psyllid population regulation and possible spread of HLB. In a preliminary study, Hu and Brlansky (2012) inoculated several non-citrus species with Las, including *Choisya* spp., *Z. fagara, A. texana* and *E. berlandieri,* via psyllids and/or grafting, but none of these species could serve as alternative transmission hosts, even though they tested positive for the pathogen. However, Lee et al. (2015) showed that *D. citri* adults can infect their feeding sites on flush shoots of *Murraya* spp. and the next generation of nymphs can acquire the pathogen through these feeding sites even though *Murraya* plants rarely seem to retain CLas pathogens. The importance of these newly identified *D. citri* host plants in the overall epidemiology of HLB is hard to predict. Inasmuch as these landscape ornamentals can serve as *D. citri* host plants, they can also be reservoirs for its natural enemies and contribute to the biological control of the pest; however, their status as reservoirs or multipliers of the pathogens via infectious colonization remains unknown.

Further research is needed on the suitability of these native species to support growth of *Ca*. L. asiaticus, and whether psyllids can transmit from them back to citrus. Damsteegt et al. (2010) showed that psyllids were able to transmit *Ca*. L. asiaticus to *M. exotica* and *M. exotica* and could also acquire the bacteria from these species and transmit them back to citrus. Attempts to transmit the bacterium to *B. koenigii* by psyllids were unsuccessful (Damsteegt et al. 2010). *B. koenigii* is one of the most favoured hosts plants for *D. citri* (Subandiyah et al. 2008), yet it does not appear to be a host for CLas. Thus, it is difficult to draw conclusions on the suitability of any plant to support CLas based on psyllid preferences – the bacterium can infect non-rutaceous species, *Catharantus roseus* (Garnier and Bové 1983) and *Lycoperisicon esculentum* (Duan et al. 2008) when transmitted by dodder.

In summary, this study revealed that some North American Rutaceae species exhibited complete resistance to *D. citri* colonization and reproduction based on reduced adult survivorship and unsuccessful nymphal development (antibiosis), or non-acceptance for oviposition (antixenosis). In contrast, three additional plants, namely *H. parvifolia*, *C. arizonica*, and *C. ternata*, have been for the first time identified to serve as hosts for *D. citri*. The various physical and chemical characteristics of these Rutaceae plants affecting their suitability for *D. citri* and possibly *Ca*. L. asiaticus need to be further investigated. Specifically, factors controlling the antixenosis and antibiosis expressed by some North American Rutaceae towards *D. citri* could help develop strategies for psyllid control and resistance against HLB. In addition, area-wide surveys need to be conducted to determine the presence of *D. citri* on any of these native rutaceous plants in future research.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Rutacaeous plant species used for *Diaphorina citri* host range testing in Texas