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HOST SUITABILITY OF CITRUS AND *ZANTHOXYLUM* SPP. FOR *LEURONOTA FAGARAE* BURCKHARDT AND *DIAPHORINA CITRI* KUWAYAMA (HEMIPTERA: PSYLLOIDEA)

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ABSTRACT

Leuronota fagarae Burckhardt (Hemiptera: Psylloidea), an exotic psyllid described from South America, was first observed in 2001 on a citrus relative *Zanthoxylum fagara* (L.) Sarg. (Sapindales: Rutaceae) in southern Florida. *Diaphorina citri* Kuwayama (Hemiptera: Psylloidea) is principal vector of the bacteria ‘*Candidatus Liberibacter* spp.’ causal agent of huanglongbing (HLB) or citrus greening disease. Both vector and disease are now well established in Florida and also reported throughout the Americas and Asia. The host range of *D. citri* is limited to citrus and some rutaceous relatives. Additional vectors and host plants could accelerate spread of HLB in citrus and threaten endangered species such as *Zanthoxylum coriaceum* A. Rich. and *Zanthoxylum flavum* Vahl. Experiments were conducted to evaluate adult survival, reproduction and nymphal development of psyllids on 3 *Citrus* and 4 *Zanthoxylum* species as well as orange jasmine, *Murraya paniculata* (Syn. *M. exotica*) (Sapindales: Rutaceae), a common ornamental and preferred host of *D. citri*. *Leuronota fagarae* in single male–female pairs at 24 °C lived an average 4–47 days, 4–12 fold longer on *Zanthoxylum* spp. (except *Z. flavum*) than on citrus. Longevity averaged 42–47 days on *Z. coriaceum*, *Z. clava-herculis* and *M. paniculata* and 17–35 days on *Z. fagara*. Average longevity on *C. sinensis*, *C. x. paradisi* and *C. reticulata* was 4–8 days. Longevity of *D. citri* in single pairs on *C. sinensis*, *C. x. paradisi*, *C. reticulata*, *Z. fagara*, *Z. flavum*, *Z. coriaceum* and *Z. clava-herculis* at 24 °C averaged 3–29 days and in cohorts on the hybrid rootstock ‘Swingle’ citrumelo and *Z. fagara* at 27 °C averaged 52–78 days. Longevities of *L. fagarae* and *D. citri* on all hosts appeared to be sufficient to acquire and transmit the causal pathogens. However, *L. fagarae* reproduced only on *Z. fagara* and *D. citri* reproduced only on citrus and *M. paniculata*, indicating that HLB spread on the inappropriate hosts would be inefficient, because acquisition of the bacteria by nymphs is much more efficient than by adults. Nevertheless, adaptation to hosts presently inappropriate for reproduction could increase over time. Field monitoring of citrus and *Zanthoxylum* spp. for both psyllid species and HLB is warranted in order to maintain effective plans for vector and disease management. The susceptibility of *Zanthoxylum* spp. to HLB pathogens and ability of *D. citri* and *L. fagarae* to acquire and transmit these pathogens are under investigation.

Key Words: endangered species, *Murraya paniculata*, Psylloidea, Rutaceae, huanglongbing, ‘*Candidatus Liberibacter*’, wild lime

RESUMEN

Leuronota fagarae Burckhardt (Hemiptera: Psylloidea), un psílido exótico descrito de América del Sur, se observó por primera vez en 2001 sobre *Zanthoxylum fagara* (L.) Sarg. (Sapindales: Rutaceae) en el sur de la Florida. *Diaphorina citri* Kuwayama (Hemiptera: Psylloidea) es el vector principal de la bacteria, ‘*Candidatus Liberibacter* sp.’ causante de la enfermedad Huanglongbing (HLB) o enverdecimiento de los cítricos. Tanto el vector como la enfermedad están bien establecidos en la Florida y están reportados a través de las Américas y Asia. La gama de hospederos de *D. citri* se limita a los cítricos y algunas rutáceas relacionadas. Otros vectores y plantas hospederas podrían acelerar la propagación del HLB en cítricos y amenazar las especies en peligro de extinción tales como *Zanthoxylum coriaceum* A. Rich. y *Zanthoxylum flavum* Vahl. Se realizaron experimentos

para evaluar la sobrevivencia de los adultos, la reproducción y el desarrollo de las ninfas de psílidos en 3 cítricos y 4 especies de *Zanthoxylum* así como jazmín naranja, *Murraya paniculata* (Syn. *M. exotica*) (Sapindales: Rutaceae), un ornamental común hospedero preferido de *D. citri*. *Leuronota fagarae* en pares individuales de un macho y una hembra en 24 °C vivió un promedio de 4-47 días, 4-12 veces más tiempo en *Zanthoxylum* spp. (excepto *Z. flavum*) que en los cítricos. El promedio de la longevidad fue de 42 a 47 días en *Z. coriaceum*, *Z. clava-herculis* y *M. exotica* y 17-35 días en *Z. fagara*. El promedio de longevidad en *C. sinensis*, *C. x. paradisi* y *C. reticulata* fue 4-8 días. El promedio de la longevidad de *D. citri* en los pares individuales en *C. sinensis*, *C. x. paradisi*, *C. reticulata*, *Z. fagara*, *Z. flavum*, *Z. coriaceum* y *Z. clava-herculis* a 24 °C fue 29.3 días y en cohortes sobre los portainjertos híbridos citromelo y *Z. fagara* 'Swingle' a los 27 °C fue un promedio de 52-78 días. La longevidad de *L. fagarae* y *D. citri* en todos los hospederos parecían ser suficiente para adquirir y transmitir los patógenos causales. Sin embargo, *L. fagarae* sólo se reproduce en *Z. fagara* y *D. citri* sólo en cítricos y *M. paniculata*, lo que indica que la diseminación de HLB en los hospederos inadecuados sería ineficaz, debido a la adquisición de las bacterias por ninfas es mucho más eficiente que por los adultos. Sin embargo, la adaptación a los hospederos actualmente inadecuados para la reproducción podría incrementar con el tiempo. El monitoreo de los cítricos y *Zanthoxylum* spp. para ambas especies de psílidos y HLB se justifica a fin de mantener los planes eficaces para manejo de vectores y la enfermedad. La susceptibilidad de *Zanthoxylum* spp. a HLB patógenos y la capacidad de *D. citri* y *L. fagarae* para adquirir y transmitir estos patógenos están bajo investigación.

Palabras Clave: especies en peligro de extinción, *Murraya paniculata*, Psylloidea, Rutaceae, huanglongbing, 'Candidatus Liberibacter', lima silvestre

Leuronota fagarae Burckhardt 1988 (Hemiptera: Psylloidea), from Paraguay, South America, was identified for the first time in North America at a nursery in Sarasota, Florida in July 2001, on wild lime, *Zanthoxylum fagara* (L.) Sarg. This was the first discovery of this species since the original description in 1988 from 12 adults collected from *Fagara rugosa* (Fiebrig) in 2 locations in Paraguay (Burckhardt 1988). Subsequent surveys after the discovery of this insect revealed that it was present on *Z. fagara* in southeastern Florida. Damage consists of rolled leaf edges that enclose the nymphs. *Leuronota fagarae* is very slender, has dark wings (Fig. 1A) and is not likely to be confused with *Diaphorina citri* Kuwayama (Hemiptera: Psylloidea), also known as the Asian citrus psyllid Fig. 1B), described from citrus *Citrus sinensis* (L.) Osbeck in Shinchiku, Taiwan in 1907 (Kuwayama 1908) or any other citrus psyllid (Halbert & Manjunath 2004).

Diaphorina citri is common throughout the citrus producing regions of Asia and now the Americas (Halbert & Manjunath 2004). *Diaphorina citri* was identified from Florida in 1998 (Halbert 1998). It is one of the known vectors of the pathogens that cause huanglongbing (HLB) or citrus greening disease and is a primary means of its spread. There are 3 species of fastidious phloem inhabiting gram-negative bacteria that are the putative causal agents of HLB: 'Candidatus Liberibacter asiaticus' (Las), 'Candidatus L. americanus' (Lam), and 'Candidatus L. africanus' (Laf) (Garnier et al. 1984; Jagoueix et al. 1996). *Diaphorina citri* vectors Las in Asia and the Americas and Lam in Brazil. The South African citrus psyllid *Trioza erythrae* (del Guercio) (Hemiptera:

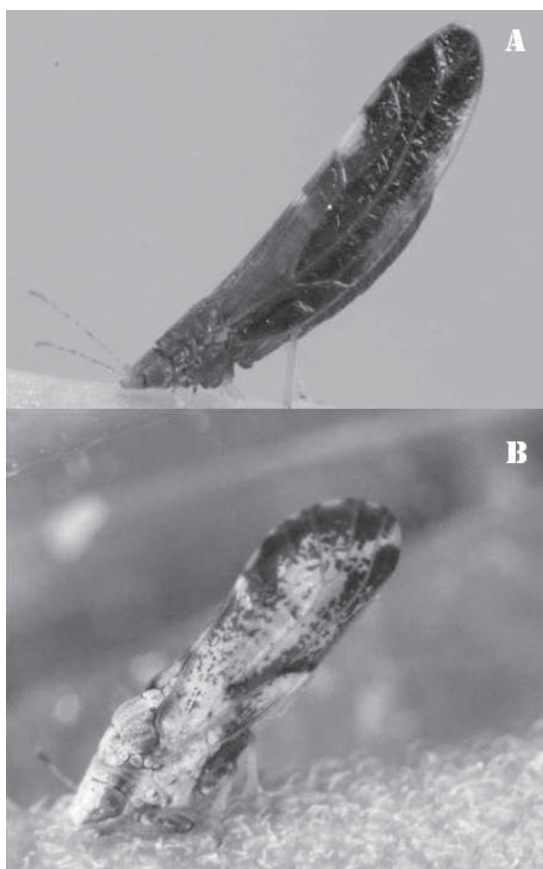


Fig. 1. Adult wild lime psyllid *Leuronota fagarae* (A, upper panel) and Asian citrus psyllid *Diaphorina citri* (B, lower panel).

Psylloidea), vectors Laf in Africa. The Asian form of HLB was identified from Florida in 2005 (Halbert 2005). Both ACP and HLB are now endemic throughout Florida (Halbert & Manjunath 2004; FDQO-CG-ACP 2008; Qureshi et al. 2009).

Citrus and its rutaceous relative *Murraya paniculata* (L.) Jack [Syn. *M. exotica* (L.) Millsp.], commonly known as orange jasmine, are suitable hosts of *D. citri* (Tsai & Liu 2000). Females need to feed on buds and shoots with unexpanded leaves to mature eggs and to oviposit (Mead 1977; Chavan & Summanwar 1993). Eggs hatch in 2-4 days and nymphs go through 5 instars in 11-15 days for a life cycle of 13-17 days at the optimum range of 25-28 °C and up to 47 days at lower temperatures (Mead 1977; Chavan & Summanwar 1993; Liu & Tsai 2000). Under controlled conditions, populations reared on *M. paniculata* at 10 °C and 33 °C failed to develop, whereas survival of nymphal instars 3-5 was unchanged between 15 and 28 °C. Highest intrinsic rate of increase ($r_m = 0.1999$) and net reproductive rate ($R_0 = 292.2$) were observed at 28 °C. Fecundity increased with increasing temperature, reaching a life time maximum of 748 eggs per female at 28 °C (Liu & Tsai 2000).

Tsai & Liu (2000) investigated the biology of *D. citri* at 25 °C on rough lemon, *Citrus jambhiri* Lush; sour orange, *C. aurantium* L.; grapefruit, *C. x paradisi* Macfayden; and *M. paniculata* in the laboratory. Average egg incubation period varied between 4.1-4.2 days on these 4 hosts. Survival of immatures on *C. paradisi*, *C. jambhiri*, *M. paniculata* and *C. aurantium* averaged 84.6, 78.3, 75.4, and 68.6%, respectively. Female adults lived an average of 39.7, 47.6, 39.7 and 43.7 days on these respective host plants. The average of 858 eggs per female on grapefruit was more than on other hosts.

Non-citrus hosts could serve as reservoirs of disease, so knowledge of the host range of the vectors and pathogens is important to help contain disease spread. Additionally, some potential rutaceous HLB host plants are endangered or threatened in Florida and may require protection if they are to be preserved. *Leuronota fagarae* was not observed during survey of citrus growing in close proximity to *Z. fagara* trees heavily infested with *L. fagarae* (Halbert & Manjunath 2004). Adults and a small nymphal colony of *D. citri* have been found during field surveys on *Z. fagara* (Halbert & Manjunath 2004). No *D. citri* have been found on other species of *Zanthoxylum* in Florida. In Florida, the genus *Zanthoxylum* contains 5 native rutaceous plants: *Z. clava-herculis* L., *Z. americanum* Mill., *Z. coriaceum* A. Rich. *Z. fagara*., and *Z. flavum* Vahl. *Zanthoxylum flavum* and *Z. coriaceum* are on the State's endangered plant list (Wunderlin & Hansen 2010). *Zanthoxylum americanum* is rare in Florida, and to our knowledge, it has never been surveyed for psyllids.

The unfortunate appearance of HLB into Florida potentially increased the importance of *L. fagarae*. *Diaphorina citri* from citrus could disperse to *Zanthoxylum* during dormant season when mature citrus trees do not produce new growth or simply use *Zanthoxylum* as a transition host to survive when moving between citrus plantings. This feeding activity potentially could transmit Las. Theoretically, *L. fagarae* might spread the bacteria among *Z. fagara* plants. Moreover, if *L. fagarae* could live on either of the endangered *Zanthoxylum* species, and they were susceptible to HLB pathogens, these native plants could be further endangered by the disease.

The objectives of this study were to determine 1) whether *L. fagarae* and *D. citri* adults could feed on the evaluated species of citrus and *Zanthoxylum* long enough to acquire or transmit the pathogens that cause HLB, 2) if *L. fagarae* could develop on citrus or on other species of *Zanthoxylum* besides *Z. fagara*, and 3) whether *D. citri* could develop and reproduce on Florida species of *Zanthoxylum*. Finally, we wanted to describe the biology of *L. fagarae*, an insect that has not been studied at all until now.

MATERIALS AND METHODS

Sources of *Zanthoxylum* and *Citrus*

Six *Z. clava-herculis* plants were purchased in 1-gal (3.8 L) pots at a nursery in Davenport, Florida, 6 *Z. coriaceum* were obtained in 3-gal (11.4 L) pots from another nursery in Palmetto Bay, Florida, and 10 *Z. flavum* were purchased in 3-gal (11.4 L) pots from Homestead, Florida. Three *M. paniculata* in 3-gal (11.4 L) pots were obtained from the Biological Control Laboratory at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI). *Zanthoxylum fagara* were grown from cuttings made from 3 plants obtained from a nursery in Gainesville, Florida. 'Duncan' grapefruit (*C. x paradisi*), 'Madam Vinous' sweet orange (*C. sinensis*) and 'Ponkan' mandarin (*C. reticulata* Blanco) were grown from seeds in the greenhouse at the DPI. Seedlings and cuttings of these plants were placed in 4.0- by 19.5-cm plastic forestry tubes ("Conetainers" [Steuwe and Sons, Corvallis, Oregon, USA]) and inserted into a Conetainer rack. The Conetainers were filled with 165 mL (133 g) of commercial readymade growing medium ("Metro-Mix 300 series"), SUN GRO Horticultural Distribution Inc., Washington, USA). The growing medium contained 30-40% horticultural grade vermiculite, plus bark, Canadian sphagnum peat moss, horticultural grade perlite, bark ash, dolomitic limestone and a wetting agent. Approximately 2 to 3 seedlings germinated from single seeds planted in each conetainer. Seedlings were thinned to one per pot after establishment.

Seedlings were fertilized with Osmocote® 4 weeks after germination and thereafter as needed.

Colonies of *Leuronota fagarae* and *Diaphorina citri*

Leuronota fagarae and *D. citri* were maintained at DPI, Gainesville, Florida, and University of Florida Southwest Florida Research and Education Center (UF-SWFREC), Immokalee, Florida. Colonies of *L. fagarae* were initiated from collections made from *Z. fagara* plants in Collier County, Florida. Adults and nymphs were caged on tall *Z. fagara* plants grown in 5-gal (19-L) pots using sleeve cages made from fine mesh organdy. Colonies of *D. citri* were maintained on 1-2 feet (30-60 cm) tall potted *M. paniculata* plants covered with screened cages. Colonies of both psyllids were kept in separate glass houses maintained at around 27 °C and 60% RH.

Evaluation of Citrus and *Zanthoxylum* as Hosts for *L. fagarae* and *D. citri*

Separate no-choice experiments with *L. fagarae* and *D. citri* were conducted at DPI to evaluate response of single male female pairs to different host plants. Both psyllids were evaluated on 3 citrus species *C. sinensis* ('Madam Vinus'), *C. x. paradisi* ('Duncan' grapefruit) and *C. reticulata* ('Ponkan' mandarin) and 4 *Zanthoxylum* species *Z. fagara*, *Z. flavum*, *Z. coriaceum* and *Z. clava-herculis*. *Leuronota fagarae* also was evaluated against *M. paniculata*. *Leuronota fagarae* came from a colony on *Z. fagara* and *D. citri* from a colony on *M. paniculata*, and were 1-week old when experiments were initiated. Both experiments were designed as randomized complete blocks (RCB) with 4 replications and conducted at 24 °C.

A single pair of male and female *L. fagarae* was placed in each Conetainer planted with *C. sinensis*, *C. x. paradisi*, *C. reticulata* or *Z. fagara* and covered with a transparent cylindrical tube. Tubes were then covered with pieces of stretchy nylon mesh secured with a rubber band to prevent the insects from escaping. *Zanthoxylum clava-herculis*, *Z. coriaceum*, *Z. flavum* and *M. paniculata* were larger potted plants so psyllids were confined to single shoots in cages made from plastic cups and fine mesh organdy.

All shoots had young emerging leaves suitable for psyllid development and reproduction. The same procedure was used for experiment with *D. citri*. Insects were observed daily for survival and progeny. Dead psyllids were removed using a soft camel's hair brush and gender determined by observing the genitalia under a stereomicroscope. Newly emerged F1 progeny adults were identified on the basis of wing color which is much lighter compared with older adults.

Development and Reproduction of *D. citri* on *Z. fagara*

Three experiments were conducted at UF-SWFREC to compare survival, development and reproduction of *D. citri* on the experimental host *Z. fagara* with 'Swingle' citrumelo and *M. paniculata* as controls. Four plants each of *Z. fagara* and 'Swingle' citrumelo were used in the first and second experiments. *Zanthoxylum fagara* plants were 4 feet (120 cm) tall in 5-gal (19-L) pots placed on the ground in a glasshouse maintained at around 27 °C and 60% RH. 'Swingle' citrumelo plants were 1.5-two feet (60 cm) tall in 1-gal (3.8-L) pots and placed on tables raised to the same height as *Z. fagara*. In the first experiment, 2 groups of adults that emerged on *M. paniculata* were evaluated for survivorship and reproduction on experimental plants. One group was allowed to feed upon emergence on *M. paniculata* for 3 d and the other for 1 week to increase the likelihood eggs would mature. Two shoots with emerging, unexpanded leaves suitable for development and reproduction of *D. citri* were confined individually on each plant using sleeve cages made from fine mesh organdy. Cohorts of *D. citri* adults containing an average 5 males and 4 females aged 3 days or 1 week were released in either cage and observed daily or every other day for survival and progeny. Dead psyllids were removed at each observation using the soft camel's hair brush and gender determined under a stereomicroscope. Once fifth instar nymphs were observed in any cage, parent psyllids were moved to another suitable shoot on the same plant and confined using a similar sleeve cage. Their progeny remained caged and F1 adults were removed and counted as they emerged.

For the second experiment, first, third and fifth instar nymphs of *D. citri* obtained from the colony on *M. paniculata* were evaluated for survival to adulthood on *Z. fagara* and 'Swingle' citrumelo. Three shoots with newly opening leaves were selected on each of the 4 plants of each host, 1 each for first, third and fifth instar nymphs. Fifteen nymphs of the respective instar were released on each shoot and observed until they started crawling. Nymphs were removed gently from *M. paniculata* plants and placed on the experimental shoots using the soft camel's hair brush. Emerging adults were counted.

For the third experiment, adults obtained from nymphs reared on 'Swingle' citrumelo were evaluated for reproduction and progeny survival on *Z. fagara* and *M. paniculata*. Adults following emergence from each of the 3 age classes of nymphs on 'Swingle' citrumelo were divided into 6 cohorts resulting in total of 18 cohorts averaging 4-8 adults per cohort. These adults were placed in the freezer for 3-4 min to calm them for gender identification using a stereomicroscope and to make sure that sex ratio averaged 1:1. Cohorts

then were caged individually on 18 young shoots of potted *M. paniculata* plants for 2 weeks to confirm that females were ovipositing before evaluation on *Z. fagara* against *M. paniculata*. The 18 cohorts were then distributed among 6 plants: 3 each of *Z. fagara* and *M. paniculata* with 3 cohorts caged per plant on 3 separate branches. Two weeks later, cohorts from *Z. fagara* were moved to *M. paniculata* to see if they were still able to produce viable progeny. Once fifth instar nymphs were observed in any cage, parent psyllids were moved to another suitable shoot and caged using a similar sleeve. Their progeny remained caged and F1 adults were removed and counted as they emerged.

Statistical Analysis

Data fitting a normal distribution as indicated by the Univariate procedure were analyzed by analysis of variance (ANOVA) General Linear Model Procedure (GLM) at a probability level of 0.05 (SAS Institute 2009, version 9.2). Mean separation for experiments with a large set of treatments was determined using Tukey's Studentized Range (HSD) test (*D. citri* or *L. fagarae* on 7 to 8 hosts) and for fewer comparisons using Least Significant Difference (LSD) test (*D. citri* only on 'Swingle' citrumelo, *M. paniculata* and *Z. fagara*) contingent on a significant F value for treatment effect. Non-normal data was analyzed using the non-parametric Kruskal-Wallis (SAS Institute, 2009, version 9.2).

Figures 1-5 are displayed in color in supplementary material for this article online in Florida Entomologist 97(4) (December 2014) at <http://purl.fcla.edu/fcla/entomologist/browse>.

RESULTS

Biology of *L. fagarae*

Adults. The adult wild lime psyllid (Fig. 1A) is a small, brown-winged insect whose body is about 3.5 mm in length, including the wings. It has a pointed face, protruding downward, bright red eyes, and short antennae. Forewings are narrow and are marked with an irregular pattern of clear to yellowish-brown patches or spots of different colors. Typically, adults can live up to 1 ½ months and can be found aggregating on new flush where they feed and mate (Fig. 2). Gravid females have a patch of orange on a portion of the abdomen when eggs are ready to be laid (Fig. 3). Each female is capable of laying several hundred eggs, depending on the host plant. The average lifespan of an adult male and female wild lime psyllid is 46 days at 20 °C.

Wild lime psyllids sit at a 45 degree angle, similar to *Diaphorina citri* Kuwayama (Fig. 1B). The camouflage is excellent. Adult psyllids look



Fig. 2. Mating pair of wild lime psyllid *Leuronota fagarae* on the underside of *Zanthoxylum fagara* leaf.

like thorns on the stems and leaves of wild lime. Females spend most of their time on the young flush. We observed that if a female is active on a flush tip, a male will feed on older tissue 5-10 cm away. If the colony is crowded, more pairs will occupy the prime feeding and egg-laying locations.

Eggs. Adult females lay tiny almond-shaped eggs, which can be yellow-orange in color (Fig. 3). Wild lime psyllids lay their eggs on the leaf margins or on both sides of the stems (Fig. 3). The anterior and posterior ends of the eggs are attached firmly to the plant. The eggs can be arranged in a line where they can either be in single, double or triple rows. They become light brown just before hatching. At 20 °C, the average egg stage takes about 7 days.

Nymphs. The nymphs are wingless, flattened, and yellow with a transparent head, thorax and tip of abdomen. They are slow crawlers with bright red eyes and produce white waxy secretion (Fig. 4). The nymphal stage spans approximately 10-12 days. The nymphs molt 4 times before the final molt into an adult.



Fig. 3. Female and eggs of wild lime psyllid *Leuronota fagarae*.

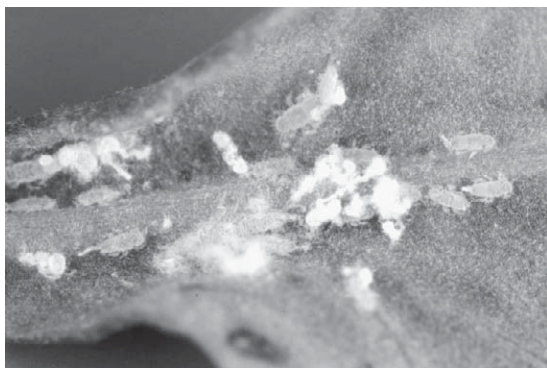


Fig. 4. Nymphs of wild lime psyllid *Leuronota fagarae* and white waxy secretions they produce.

Damage caused by wild lime psyllids is similar to damage caused by other psyllids, especially some other triozids such as *Leuronota maritima* (Tuthill) on *Avicennia* and *Trioza diospyri* (Ashmead) on *Diospyros*. Feeding results in curling and twisting of the leaves, especially rolling of the leaf margins (Fig. 5) and shoot growth is reduced.

Longevity and Reproduction of *L. fagarae*

Longevity of males differed significantly among hosts ($F = 45.32$, $df = 7, 24$, $P < 0.0001$, Table 1). Average adult lifespan on *Z. coriaceum* was 46.5 ± 0.5 days and on *Z. clava-herculis* or *M. paniculata* was 41.5 ± 3.2 days which was significantly longer compared to all other hosts including *Z. fagara* on which this psyllid was first observed in Florida. Males lived an average of 16.8 ± 6.3 days on *Z. fagara*, significantly longer than 2.3 ± 0.5 days on *Z. flavum*. Male longevity on *Z. fagara* or *Z. flavum* did not differ statistically from any of the 3 citrus species which did not differ from each other (Table 1).

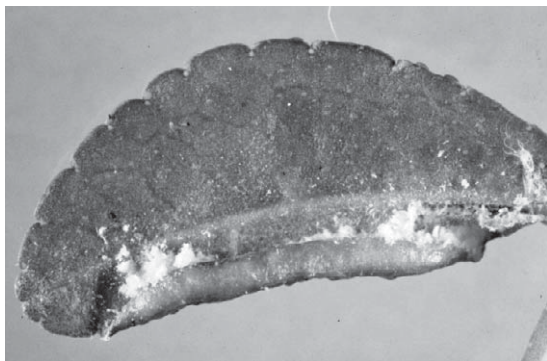


Fig. 5. Young leaf of *Zanthoxylum fagara* curled by wild lime psyllid *Leuronota fagarae*.

Females lived an average 46.5 ± 0.5 days on *Z. coriaceum* which was similar to 41.5 ± 3.2 days on *Z. clava-herculis* and *M. paniculata* but significantly longer than on other hosts, including 35.00 ± 0.0 days on *Z. fagara* ($F = 107.51$, $df = 7, 24$, $P < 0.0001$, Table 1). Longevity was statistically similar among *Z. clava-herculis*, *M. paniculata* and *Z. fagara* but greater than *Z. flavum* or any of the 3 citrus species (Table 1).

Females lived as long or longer than males. Female longevity was double that of males on *C. sinensis*, *C. x. paradisi* and *Z. fagara*. F1 adults were recovered only on *Z. fagara* and averaged 26.3 ± 6.3 days per female.

Longevity and Reproduction of *D. citri*

Experiments Using Male Female Single Pairs

Male longevity was significantly affected by the host plant ($F = 5.06$, $df = 6, 21$, $P = 0.0034$, Table 1). Males lived an average of 29 ± 0.0 days on *Z. clava-herculis* significantly longer compared with all other hosts except 16.5 ± 7.3 days on *C. sinensis* and 10.5 ± 1.8 days on *Z. coriaceum*. There was no difference between these later two and the remaining hosts. The shortest lived males with an average life of 2.8 ± 1.8 days were observed on *C. reticulata* (Table 1). For some reason, our mandarin plants were not attractive for colonization, possibly due to the growth stage used.

Female longevity was also significantly affected by host plant ($F = 6.45$, $df = 6, 21$, $P = 0.0009$, Table 1). Longest lived females were observed on *Z. clava-herculis* with an average of 29 d, statistically similar to 19.3-20 days on *Z. flavum*, *C. sinensis* or *C. x. paradisi* but significantly longer compared with an average of 4-8 days on *Z. fagara*, *Z. coriaceum* and *C. reticulata*. The shortest lived females, averaging 3.8 ± 1.3 days of life, were observed on *Z. fagara*. F1 progeny matured on only *C. sinensis* and *C. x. paradisi*, resulting in 4.5 ± 0.5 and 3.5 ± 0.5 adults per female, respectively.

Effects of host plant on longevity of the longest lived individual were significant ($F = 5.23$, $df = 6, 21$, $P = 0.0028$). Generally, females lived the same or longer than males except on *Z. fagara* and *Z. coriaceum* (Table 1). Therefore, means and differences among means for longest lived individual were same as for females (Table 1).

Experiments Using Cohorts

Adult *D. citri* Longevity. Adults transferred to either *Z. fagara* or Swingle citrumelo lived an average of 68.7 ± 3.4 days and 64.2 ± 3.5 days following 3 days or one week on *M. paniculata*, respectively with no significant effect of time on the first host ($F = 0.83$, $df = 1, 6$, $P = 0.3695$). However, final host plant effects were significant with

TABLE 1. MEAN (\pm SEM) LONGEVITY (DAYS) OF MALE (δ) AND FEMALE (φ) *LEURONOTA FAGARAE* AND *DIAPHORINA CITRI* ADULTS ON DIFFERENT PLANT SPECIES WHEN TESTED BY SINGLE MALE AND FEMALE PAIRS. *LEURONOTA FAGARAE* USED IN THE TESTS WERE REARED ON *ZANTHOXYLUM FAGARA* AND *DIAPHORINA CITRI* ON *MURRAYA PANICULATA* AND WERE ONE WEEK OLD WHEN EXPERIMENTS WERE INITIATED.

Treatment	<i>L. fagariae</i> δ (plant ⁻¹)	<i>L. fagariae</i> φ (plant ⁻¹)	<i>D. citri</i> δ (plant ⁻¹)	<i>D. citri</i> φ (plant ⁻¹)
<i>C. sinensis</i>	3.5 \pm 0.9 bc	7.5 \pm 1.8 c	16.5 \pm 7.3 ab	19.5 \pm 5.6 ab
<i>C. x. paradisi</i>	3.5 \pm 0.9 bc	6.8 \pm 1.4 c	8.8 \pm 6.8 b	20.0 \pm 5.3 ab
<i>C. reticulata</i>	3.5 \pm 0.7 bc	4.0 \pm 0.4 c	2.8 \pm 1.1 b	7.5 \pm 2.3 b
<i>Z. fagara</i>	16.8 \pm 6.3 b	35.00 \pm 0.0 b	4.00 \pm 1.5 b	3.8 \pm 1.3 b
<i>Z. flavum</i>	2.3 \pm 0.5 c	2.3 \pm 0.5 c	9.0 \pm 1.4 b	19.3 \pm 3.6 ab
<i>Z. coriaceum</i>	46.5 \pm 0.5 a	46.5 \pm 0.5 a	10.5 \pm 1.8 ab	6.0 \pm 1.5 b
<i>Z. clava-herculis</i>	41.5 \pm 3.2 a	41.5 \pm 3.2 ab	29.0 \pm 0.0 a	29.0 \pm 0.0 a
<i>M. paniculata</i>	41.5 \pm 3.2 a	41.5 \pm 3.2 ab	*	*

Means within column followed by the same letter are not significantly different (Tukey's HSD, $P > 0.05$)

**M. paniculata* was not used in the *D. citri* experiment

longevity averaging 76.2 \pm 1.5 d and 56.7 \pm 3.1 days on 'Swingle' citrumelo and *Z. fagara*, respectively ($F = 35.53$, $df = 1, 6$, $P < 0.0001$). Males and females on 'Swingle' citrumelo lived significantly longer than their counterparts on *Z. fagara* (Male: $F = 36.09$, $df = 1, 6$, $P < 0.0001$; Female: $F = 10.76$, $df = 1, 6$, $P = 0.0073$, Fig. 6) with a difference of 23 days and 16 days, respectively. All males survived through 13 days on 'Swingle' citrumelo compared to 8 days on *Z. fagara*, decreasing to 76% and 31%, respectively at 82 days (Fig. 7A). Female survival was 100% through 38 days on 'Swingle' citrumelo but only 10 days on *Z. fagara* and 82% and 55%, respectively, at 82 days (Fig. 7B).

No F1 *D. citri* adults were recovered from cohorts on *Z. fagara* compared with 57 \pm 14 per female on 'Swingle' citrumelo during 4-5 weeks after the move from *M. paniculata* ($\chi^2 = 12.89$, $df = 1$, $P = 0.0003$). Cohorts with initial exposure of 3 days or 1 week on *M. paniculata* produced an average of 31.8 \pm 5.5 and 82.3 \pm 21.4 adults per female, respectively, on 'Swingle' citrumelo, without significant effect of the time of initial exposure ($F = 4.02$, $df = 1, 6$, $P = 0.1388$).

Nymphal *D. citri* Survival. Nymphs of *D. citri* released on *Z. fagara* did not develop to adulthood. However, emergence of adults from first, third and fifth instar nymphs released on 'Swingle' citrumelo averaged 61.3 \pm 6.4%, 68.3 \pm 7.4% and 83.3 \pm 4.3%, respectively.

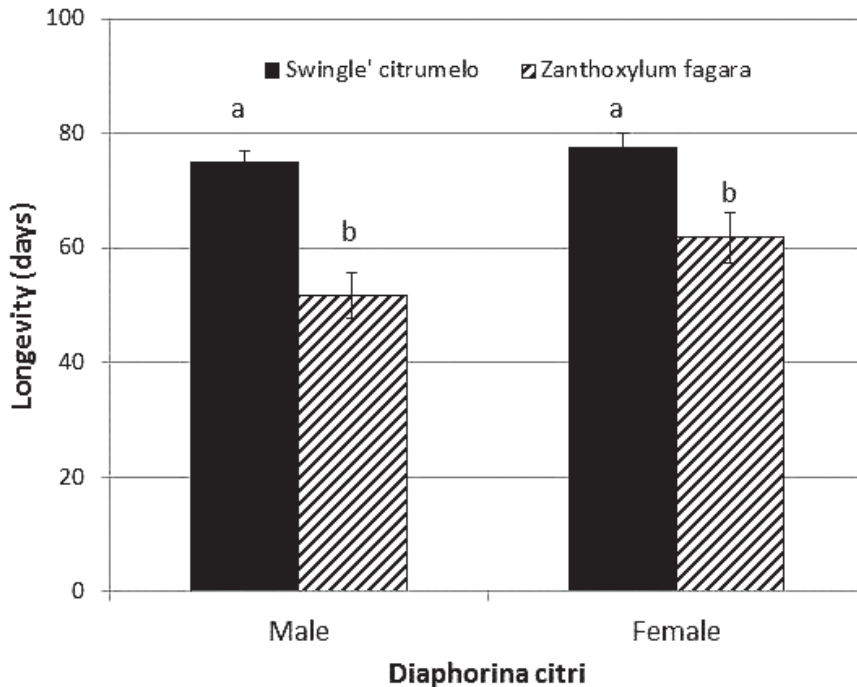
Effects of Host-Nonhost Transfers. Cohorts of *D. citri* adults reproducing actively on *M. paniculata* produced an average of 1.04 eggs per female during 2 weeks after being transferred to *Z. fagara* compared with parallel cohorts on *M. paniculata* which produced 19.02 eggs per female (Table 2). Nymphs hatching on *Z. fagara* during this period did not develop into adults, compared with 24.9 \pm 8.9 per female that did so over the same interval on *M. paniculata*. When cohorts on *Z. fagara* were moved to *M. paniculata*, reproduc-

tion was similar to individuals left on *M. paniculata*, 51-55 adults per female (Table 2).

DISCUSSION

Leuronota fagariae adults lived longer on *Z. coriaceum*, *Z. clava-herculis* and *Z. fagara* than on citrus with an average difference of 4 to 12-fold (Table 1). This result indicated less ability of *L. fagariae* to utilize citrus as an adult host than that of *D. citri* to utilize *Zanthoxylum*. Additionally, *Z. fagara* was the only host on which *L. fagariae* could reproduce. In fact, high reproductive potential of *L. fagariae* as observed on *Z. fagara* in colonies could cause serious damage just from the feeding of developing nymphs (JA Qureshi, personal observation). Populations in the field probably are impacted by predators and parasitoids and therefore likely to be lower than the levels observed in the colonies. In fact, it is rare to find thriving colonies of naturally occurring *L. fagariae* (S. Halbert, personal observation).

Successful survival of *D. citri* on 4 *Zanthoxylum* species (*Z. fagara*, *Z. flavum*, *Z. coriaceum* and *Z. clava-herculis*) as well as known citrus hosts *C. sinensis*, *C. x. paradisi* and *C. reticulata* and *M. paniculata*, indicates significant potential of adults to utilize other rutaceous hosts when necessary. Field surveys in Florida show that all 3 citrus species that we evaluated are common hosts of *D. citri* and Las (Aubert 1987, 1990; Tirtawidjaja 1981; Miyakawa & Yuan 1990; Koizumi et al. 1996; Halbert & Manjunath 2004). A growth chamber study conducted by Tsai & Liu (2000) at 25 °C showed *C. x. paradisi* was a preferred host of *D. citri*, with average female longevity of 39.7 d, 858 eggs and 84% nymphal survival. In our experiment conducted at 24 °C using single pairs, no significant difference in longevity of males or females was observed among all 3 species of citrus. Female longevity on *C. x. paradisi* averaged 20 days, although only 3.5 nymphs per female



Columns representing means with different letters differ significantly for male or female (LSD, $P < 0.05$)

Fig. 6. Mean (\pm SEM) longevity of male (δ) and female (φ) *Diaphorina citri* adult on 'Swingle' citrumelo and *Zanthoxylum fagara*. *Diaphorina citri* used in the tests were reared on *M. paniculata* and were 3 days or one week old when experiments were initiated. No significant effect of time adults spent on *M. paniculata* on their longevity on 'Swingle' citrumelo and *Z. fagara* was observed, therefore, data were combined.

matured to adulthood, probably because of the cramped conditions and limited suitable growth for nymphal development. Difference in findings with Tsai & Liu (2000) could be due to the variability in conditions between growth chamber and room and study arenas, and the availability of one vs. 2 males to each female which could have impacted the reproductive performance of females.

Liu & Tsai (2000) observed mean longevity of *D. citri* increase with increasing temperature within 15-28 °C range. They also reported the highest intrinsic rate of increase (0.199) and net reproductive rate (292.2) for populations reared at 28 °C. In our study with caged cohorts of *D. citri* on 'Swingle' citrumelo at 27 °C males and females lived an average 75-78 d, longer compared with average of less than 47.6 d at 24-25 °C that we or Tsai & Liu (2000) observed. Reproductive performance of females also improved in the experiment conducted at 27 °C compared to 24 °C with 32 to 82 nymphs maturing to adulthood per female during 4-5 weeks after transfer from *M. paniculata* to 'Swingle' citrumelo. These findings

indicate that in addition to more ventilated arenas and chances to mate, temperature could be another important factor that contributed to enhanced longevity and reproduction in the cohort experiments.

Halbert & Manjunath (2004) mention no reports of *D. citri* presence on *Z. clava-herculis* or any other *Zanthoxylum* except occasional presence on *Z. fagara*. In that study, the 2 *Z. clava-herculis* plants in the Florida Department of Agriculture and Consumer Services, Division of Plant Industry Citrus Arboretum were surveyed each time other citrus relatives at the Arboretum were examined. Occasional adult *D. citri* were observed on *Z. fagara* plants in both the Arboretum and in Miami (S. Halbert, personal observations). We did not observe significant differences in the longevity of *D. citri* on *Z. fagara*, *Z. flavum*, *Z. coriaceum*, *C. sinensis*, *C. x. paradisi* and *C. reticulata* in the experiment that tested single pairs at 24 °C. In fact, adults lived longer on *Z. clava-herculis* than *C. x. paradisi*, *C. reticulata*, *Z. fagara*, *Z. flavum* and *Z. coriaceum* (Table 1). This result indicated significant potential of this pest

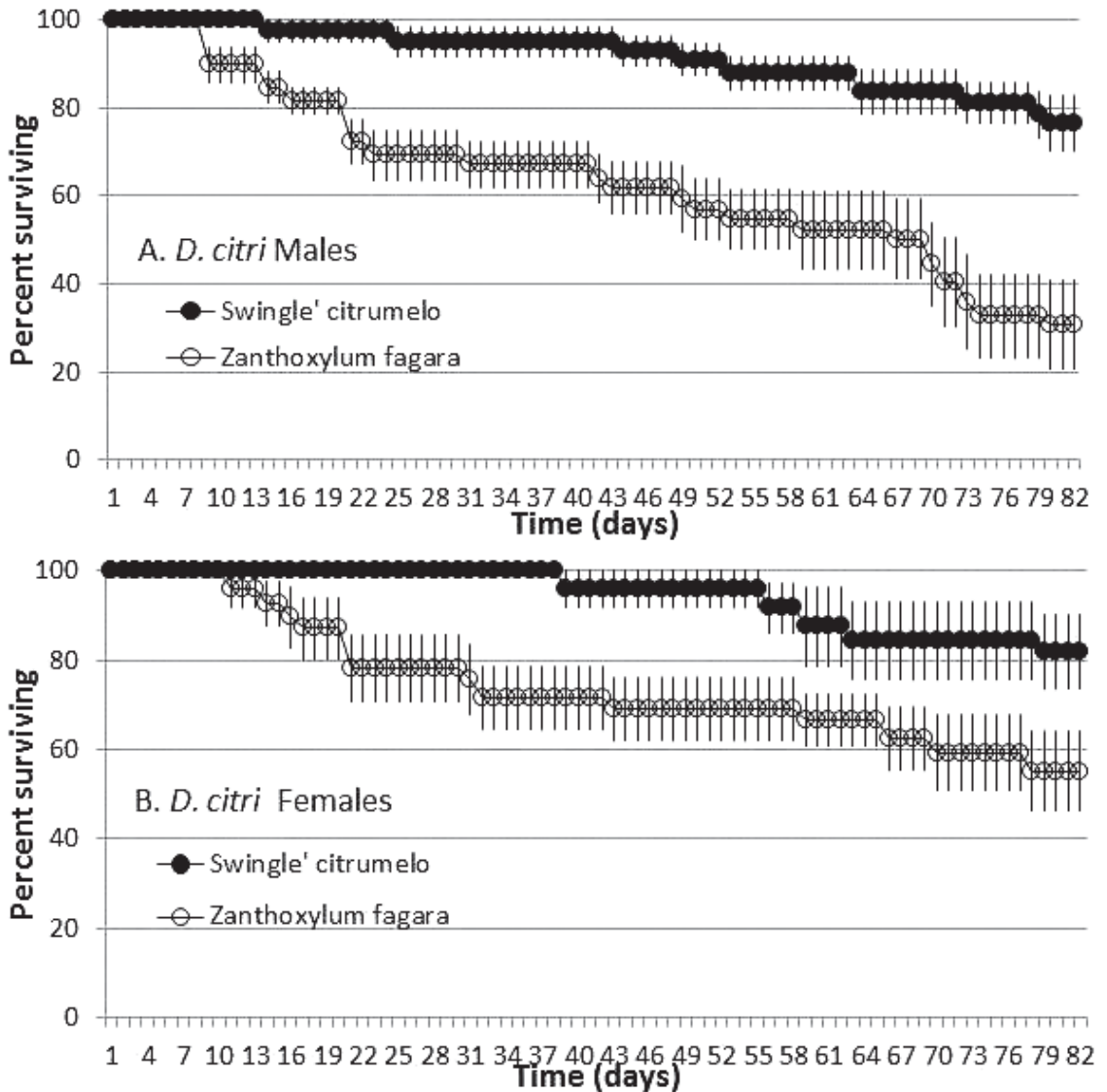


Fig. 7. Mean (\pm SEM) cumulative percentage survival of male (δ) and female (η) *Diaphorina citri* adult on 'Swingle' citrumelo and *Zanthoxylum fagara*. *Diaphorina citri* were reared on *M. paniculata* and were 3 day or one week old when experiments were initiated. No significant effect of time adults spent on *M. paniculata* on their longevity on Swingle citrumelo and *Z. fagara* was observed, therefore, data were combined.

to utilize all these hosts for survival when preferred hosts are not available. Further evidence of this was observed in the cohort experiment when average longevity of 52-62 d were observed on *Z. fagara*, although significantly less than 72-78 d observed on 'Swingle' citrumelo (Figs. 6 and 7).

Diaphorina citri did not reproduce on any of the 4 *Zanthoxylum* species when young females were tested and eggs and nymphs were minimal when already reproducing females were moved to *Z. fagara* (Tables 1 and 2). Young and mature nymphs of *D. citri* that were moved from *M. paniculata* to *Z. fagara* also died, indicating that

nymphal survival must be minimal at best, in spite of occasional finds of *D. citri* nymphs on *Z. fagara* (Halbert & Manjunath 2004). However, all *Zanthoxylum* evaluated in this study appear to support adult life similar to citrus, up to 2 months on *Z. fagara* (Fig. 6). Adults were able to retain their reproductive potential on *Zanthoxylum* and oviposited effectively and produced viable nymphs when moved back to preferred host *M. paniculata* at rate similar to adults which stayed on *M. paniculata* (Table 2). These findings indicate that *D. citri* may be able to use *Zanthoxylum* as a transition host when moving between citrus plantings

TABLE 2. MEAN (± SEM) PROGENY OF *DIAPHORINA CITRI* ON *MURRAYA PANICULATA* AND *ZANTHOXYLUM FAGARA* USING COHORTS OF 4-8 ADULTS AND SEX RATIO OF 1:1. ADULTS DEVELOPED FROM NYMPHS REARED ON 'SWINGLE' CITRUMELLO AND THEN ALLOWED TWO WEEKS ON *M. PANICULATA* TO MATURE EGGS AND INITIATE OVI-POSITION BEFORE EVALUATION ON *M. PANICULATA* AND *Z. FAGARA*, AND AFTER OBSERVING NO VIABLE REPRODUCTION ON *Z. FAGARA* ADULTS WERE MOVED TO *M. PANICULATA* TO SEE IF THEY WERE STILL SUCCESSFUL IN PRODUCING PROGENY.

Host	Mean ± SEM numbers per female				Mean ± SEM numbers per female			
	Cumulative first and second week				Cumulative third and fourth week			
	Eggs	Nymphs	Eggs + Nymphs	Adults	Host	Adults	Adults	Adults
<i>Murraya paniculata</i>	19.02 ± 10.52 a	10.56 ± 7.31 a	29.57 ± 10.78 a	24.89 ± 8.89 a	<i>Murraya paniculata</i>	50.93 ± 10.27 a		
<i>Zanthoxylum fagara</i>	1.04 ± 0.74 b	1.96 ± 1.04 a	3.00 ± 1.35 b	0.00 ± 0.00 b	<i>Murraya paniculata</i>	55.21 ± 13.80 a		
χ²	5.4129	0.0212	7.5871	12.18		0.0019		
P	0.0200	0.8843	0.0059	0.0005		0.9648		

Means within a column followed by the same letter were not significantly different (Kruskal–Wallis, $P > 0.05$).

or for survival during winter when mature citrus produces little or no new growth required to support psyllid development and survival. Therefore, regular monitoring of *Zanthoxylum* and possibly other non-reproductive hosts for management of *D. citri* is warranted wherever large populations of such hosts occur near citrus.

Implications for transmission of Las

Studies on citrus have shown that *D. citri* acquire Las more efficiently as nymphs than adults (Capoor et al. 1974; Inoue et al. 2009; Pelz-Stelinski et al. 2010). Acquisition times of 30 min for *D. citri* (Roistacher 1991) and 24 h for *T. erytreae* have been reported (Buitendag & von Broembsen 1993). Xu et al. (1988) found that acquisition by adults feeding of 5-7 h was sufficient to transmit citrus greening pathogens, while feeding periods of 1-3 h were not. Inoue et al. (2009) reported that Las apparently multiplies in *D. citri* when acquired by nymphs but not when acquired by adults. They also suggested that multiplication of the bacterium within the psyllids is essential for efficient transmission, and that it is difficult for adults to transmit the pathogen unless they acquire it as nymphs. Pelz-Stelinski et al. (2010) found that adult *D. citri* that acquired the HLB pathogen as adults were poor vectors of the pathogen compared with adults that acquired the pathogen as nymphs. Considering high HLB infection rates in Florida citrus, most psyllid adults that end up utilizing *Zanthoxylum* as host could originate from nymphs that develop on infected trees.

Murraya paniculata is among the hosts that supported *L. fagarae* for the longest time (Table 1). However HLB symptoms or Las detection by Polymerase Chain Reaction (PCR) in *M. paniculata* is very rare in spite of its being a preferred host of *D. citri*. A host may be preferred for the vector but may not be preferred for the pathogens. For example, symptoms or PCR identification of HLB in *M. paniculata*, a preferred host to *D. citri*, are not common even in regions with high incidence of HLB (Tirtawidjaja 1981; Toorawa 1998; Hung et al. 2000; Deng et al. 2007). However, Las-positive *D. citri* nymphs have been documented on *M. paniculata* (Manjunath et al. 2008). Thus, even a non-preferred host of Las might be a source of positive psyllids.

Since acquisition of the HLB pathogen is largely the providence of nymphs, it appears to be unlikely that *Z. fagara* would ever serve as a source of Las acquired by *D. citri*. Pathogens transmitted to *Zanthoxylum* species by *D. citri* coming from infected citrus would be stuck in a dead end host unless *L. fagarae* was able to transmit Las among *Z. fagara* plants. Whether *Zanthoxylum* spp. plant would suffer from the disease is yet to be determined. In any case it seems unlikely

that either *L. fagariae* or any *Zanthoxylum* species would present much of a threat for HLB spread to citrus, especially considering the relative rarity of these plants.

Diaphorina citri female adults survived on *Z. flavum* for 19 days, and on *Z. coriaceum* for 6 days, which is long enough for transmission of Las to these endangered plants. Their susceptibility to HLB is under investigation. If *Z. flavum* or *Z. coriaceum* did become infected, they are likely to be dead end hosts, because neither *D. citri* nor *L. fagariae* reproduced on them, and no other Rutaceae-feeding psyllids are known in Florida. *Zanthoxylum flavum* supported *L. fagariae* for the least amount of time compared with all other species and may be at less risk. *Diaphorina citri* females lived on *Z. fagara* up to 60 days, which is long enough for occasional transmission of Las. It remains to be seen if *L. fagariae* can acquire or transmit Las. The susceptibility of *Zanthoxylum* spp. to HLB pathogens and ability of *L. fagariae* to acquire and transmit these pathogens is under investigation.

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