Environmental Entomology, 45(4), 2016, 897–908
doi: 10.1093/ee/nvw062
Advance Access Publication Date: 20 June 2016
Research article

Fundamental Host Range of *Leptoypha hospita* (Hemiptera: Tingidae), a Potential Biological Control Agent of Chinese Privet

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Received 16 November 2015; Accepted 4 May 2016

Abstract

Chinese privet, *Ligustrum sinense* Lour., is an invasive shrub within riparian areas of the southeastern United States. Biological control is considered the most suitable management option for Chinese privet. The potential host range of the lace bug, *Leptoypha hospita* Drake et Poor, was evaluated on the basis of adult feeding and oviposition, combined oviposition–nymphal development no-choice tests, nymphal development no-choice tests, multiple generation comparison on *Forestiera pubescens* Nutt. and *L. sinense* no-choice tests, and multiple-choice tests with 45 plant species in 13 families. No-choice tests showed that the host range of *L. hospita* was restricted to the tribe Oleeae. In adult feeding and oviposition no-choice tests, the bug fed and oviposited significantly more on Chinese privet than all other test plant species except for three native *Forestiera* spp., two nonnative *Syringa* spp., and another exotic *Ligustrum* sp. Among those, only *F. pubescens* supported complete development in numbers comparable to Chinese privet. However, when reared for multiple generations lace bugs reared on *F. pubescens* were smaller and had lower fecundity than those reared on *L. sinense*, suggesting *F. pubescens* is not an optimal host. In multiple-choice tests, *L. hospita* displayed a strong preference for feeding and ovipositing on Chinese privet over other test plant species, with the exception of the closely related nonnative *Syringa* spp. and its congenic species *Ligustrum vulgare.* The results of this study suggest that the risk to nontarget plant species in North America is minimal, and *L. hospita* would be a promising candidate for Chinese privet biological control.

Key words: *Ligustrum sinense*, Oleeae, *Forestiera acuminata*, *Forestiera pubescens*, *host specificity testing*

Chinese privet, *Ligustrum sinense* Lour. (Oleaceae), is a perennial evergreen shrub native to China, Vietnam, and Laos (Wu and Raven 2003). After introduction into the United States as an ornamental in 1852 (Miller 2005), the species escaped cultivation and established throughout the southeast by 1932 (Small 1933). Since then it has continued to spread and currently occupies over one million hectares in 12 southern states based on USDA Forest Service Inventory and Analysis data (Miller et al. 2008). Chinese privet is ranked among the top 10 exotic plant pests of Georgia (Georgia Exotic Pest Plant Council, 2001) and Mississippi (Matlack 2002), and it is considered a naturalized exotic in at least 12 other countries (Invasive Species Specialist Group 2005), including Australia (Burrows and Kohen 1986), Argentina (Montaldo 1993), and New Zealand (Invasive Species Specialist Group 2005). The aggressive invader often forms monotypic stands in riparian forests, along fencerows, forest edges, and rights-of-way (Miller 2003). The expansion of Chinese privet into new areas is largely attributed to seed dispersal by birds (Miller 2003, Williams and Minogue 2008) and floodwaters (Ward 2002). Several factors contribute to its success and spread such as high growth rates, vegetative reproduction, shade tolerance, and prolific seed production (Langeland and Burkes 1998). In invaded habitats Chinese privet reduces native plant biodiversity and suppresses tree regeneration (Morris et al. 2002, Wilcox and Beck 2007, Hanula et al. 2009, Hudson et al. 2014) and causes reductions in diversity and abundance of several insect groups, including pollinators (Ulyshen et al. 2010; Hanula and Horn 2011a b; Hudson et al. 2013), and native earthworms in the soil (Lobe et al. 2014). Chinese privet also has high quality leaf litter with lower lignin, cellulose, and C: N ratios relative to native leaf litter (Brantley 2008). These differences result in faster decomposition rates relative to native litter in floodplains of western Georgia, as well as a fivefold increase in soil N mineralization rates (Mitchell et al. 2011).

Large-scale control of Chinese privet is labor-intensive and requires the use of large amounts of herbicides (Hanula et al. 2009), thus classical biological control is seen as the most practical, sustainable long-term solution. Chinese privet is a promising target for biological control because there are no native species of *Ligustrum* (USDA, NRCS 2013) or prospective natural enemies in North America (Williams and Minogue 2008). A U.S.–China cooperative biological control project was initiated in 2005 and among 170 insect species found associated with Chinese privet during an herbivore survey in China (Zhang et al. 2008a), a lace bug (Hemiptera: Tingidae: *Leptoypha hospita* Drake et Poor) and a flea beetle (Coleoptera: Chrysomelidae: *Argopistes tsekooni* Chen) showed strong potential as biocontrol agents. Both were abundant and damaging insects feeding on Chinese privet in survey areas (Zhang et al. 2008a b, 2009). Tingid species are highly specialized (Drake and Ruhoff 1965), and several have been used in biological control of invasive plants worldwide. For example, host specificity tests of *Carvalhotingis visenda* (Hemiptera: Tingidae) using 38 plant species in 10 families showed that it is a highly host specific biocontrol agent for cat's claw creeper *Macfadyena unguis-cati* (Bignoniaceae) in Australia (Dhileepan et al. 2007). Likewise, *Gargaphia decoris* (Hemiptera: Tingidae) displayed a relatively narrow host range in captivity in host specificity tests, and became the first agent to be released in South Africa for biological control of *Solanum mauritianum* Scopoli (Solanaceae) (Olckers 2000).

Both adults and nymphs of tingids pierce the epidermis and feed on intracellular contents, resulting in plasmolysis. Severe feeding causes shoot and leaf discoloration and premature leaf abscission, resulting in stunted growth of plants and reduced plant vigor (Drake and Ruhoff, 1965, Olckers 2000). Our prerelease evaluations using simulated defoliation of potted and field grown plants as well as inoculation of potted plants in the laboratory with *L. hospita* provide evidence that it has the potential to affect the growth of *L. sinense* (Zhang et al. 2013). Because *L. sinense* is an evergreen species that grows beneath deciduous tree canopies, a species that causes continuous or late season defoliation, or reduces winter photosynthetic activity, may be very effective. Both nymphs and adults of *L. hospita* feed on the leaf mesophyll cells, which leads to a bleached appearance of leaves and dieback of branch tips. Recent studies show that it can complete a generation in 25 d and females can lay an average of 240 eggs (Zhang et al. 2011). It has multiple overlapping generations each year and adults live an average of 75 d. All of these positive biological characteristics suggest that the lace bug has potential as a biocontrol agent (Zhang et al. 2011). *Leptoypha hospita* is native to southern China, Penang Island, and Malaysia (Drake and Ruhoff 1965). There is little information about its host range, even though it causes considerable damage to ornamental Chinese privet in China (Zhang Y.Z., personal observation). Thus far, *Ligustrum sinense*, *Ligustrum quihoui* Carrière, and *Ligustum obtusifolium* Siebold & Zucc. are the only recorded hosts (Li 2001). The objective of this study was to investigate the host range of *L. hospita* on plants native to the United States, as well as selected ornamental and agricultural plant species to assess its suitability for release in the United States as a biological control agent of *L. sinense*.

Materials and Methods

Insect Colony

Leptoypha hospita adults were field collected from *L. sinense* planted as an ornamental hedge in Huangshan city (29° 42′23″ N, 118° 19′05″ E), Anhui province, China, in March 2009. Adults and excised branch tips were packaged and shipped to the United States (USDA-APHIS permit P526P-08-01107). Upon arrival, lace bugs were transferred to potted Chinese privet plants in a quarantine laboratory at the University of Georgia horticultural farm near Watkinsville, GA. Chinese privet used for maintaining the colony was either purchased from nurseries and repotted in 8-liter pots, or seedlings dug from the field were planted directly into 8-liter pots of Miracle-Gro potting mix (ScottsMiracle-Gro, Marysville, OH, with the N:P:K being 3:1:2). Plants were maintained in a lath shade house at the University of Georgia's Whitehall Forest, where they were watered and fertilized as needed. Potted Chinese privet was moved to the quarantine laboratory continuously to maintain the colony. Plants were covered with white polyester cages (90 cm in height) to keep lace bugs from escaping. The lace bug colony was maintained in the quarantine laboratory by transferring bugs to new plants as old plants deteriorated. Lace bugs used in this study all came from this colony, which was maintained at 24–26°C, 50–80% RH, and a photoperiod of 15:9 (L:D) h.

Test Plant List

The test plant list was developed according to the modified centrifugal phylogenetic method (Wapshere 1974, Briese 2003), and revised adopting recommendations from the Federal interagency Technical Advisory Group for Biological Control Agents of Weeds (TAG), and plant availability. *Ligustrum sinense* belongs to the order Lamiales, family Oleaceae, tribe Oleeae, and subtribe Ligustrinae. Lamiales, previously the Scrophulariales, has undergone one of the largest revisions resulting from the adoption of the APG II classification as new molecular phylogenetic evidence has emerged (The Angiosperm Phylogeny Group 2003, Olmstead et al. 2001). The current test plant list (Table 1) was based more on true phylogenetic relationships rather than taxonomic nomenclature. The list was compiled starting with the target weed (*L. sinense*) and adding representative species based on categories recommended by TAG.

Test plants were purchased from various nurseries in several states and were maintained in the same lathe shade house as Chinese privet. All plants were kept in this lathe house for a minimum of three months to mitigate the chances that insecticides had been applied by nurseries. Chinese privet used for host specificity tests were those dug from the field and grown by us. All plants were fertilized using Miracle-Gro Shake 'n Feed Continuous Release All Purpose Plant Food (ScottsMiracle-Gro, Marysville, OH) and watered as needed.

Experimental Design

The design of the host specificity tests was based on the testing sequence of potential biocontrol agents for weeds method proposed by Wapshere (1989). Because van Klinken's (2000) proposed experimental methods of host specificity tests for biocontrol agents can be modified based on biological characteristics and life history of the focal insect, we chose to test adult feeding and oviposition preference first. For *L. hospita*, whose nymphs have limited mobility, host selection mainly depends on adult oviposition preference (Zhang et al. 2011). Nymphal development no-choice tests were conducted if oviposition occurred. All tests were conducted in the same quarantine laboratory and under the same ambient conditions as the lace bug colony.

Adult Feeding–Oviposition, and Combined Oviposition–Nymphal Development No-Choice Tests

Because all plant species could not be tested simultaneously due to space limitations, plants were tested randomly in a series of 13

Table 1. Classification of testing plants

Order	Family[a]	Tribe[b]	Subtribe[b]	Species
Category 1: Species in the same genus as the target weed				
Lamiales	Oleaceae	Oleeae	Ligustrinae	*Ligustrum vulgare* L.
				Ligustrum japonicum Thunb.
Category 2: North American species in other genera in the same family as the target weed				
Category 2A: species in the same subtribe (Ligustrinae) as the target weed				
				Syringa meyeri C.K. Schneid.
				Syringa pubescens 'Miss Kim'
				Syringa oblata Lindl.
				Syringa vulgaris L.
Category 2B: species in different subtribes in the same tribe (Oleeae)				
			Fraxininae	*Fraxinus americana* L.
				Fraxinus caroliniana Mill.
				Fraxinus nigra Marshall
				Fraxinus pennsylvanica Marshall
				Fraxinus quadrangulata Michx.,
				Fraxinus profunda (Bush) Bush
			Oleinae	*Chionanthus pygmaeus* Small
				Chionanthus virginicus L.
				Forestiera acuminata (Michx.) Poir.
				Forestiera godfreyi L.C. Anderson
				Forestiera pubescens Nutt. var. *pubescens*
				Forestiera segregata (Jacq.) Krug & Urb.,
				Olea europaea L.
				Osmanthus americanus (L.) Benth. & Hook. f. ex A. Gray
				Osmanthus fragrans Lour.
Category 2C: species in different tribes in the same subfamily/family				
		Forsythieae	N/A	*Forsythia* × *intermedia* Zabel
				Fontanesia fortunei Carr.
				Jasminum nudiflorum Lindl.
Category 3: Threatened, endangered, or sensitive species in the same family as the target weed				
		Oleeae	Oleinae	*Chionanthus pygmaeus* Small
Category 4: Species in other families in the same order as the target weed (Lamiales)				
	Buddlejaceae	Buddlejeae	Buddlejinae	*Buddleja* × *weyeriana*
			Buddlejinae	*Buddleja cordata* ssp. *tomentella* (Standl.) Norman
	Lamiaceae	Mentheae	Menthinae	*Monarda didyma* L.
			Salvinae	*Salvia microphylla* Benth.
			Salvinae	*Perovskia atriplicifolia* Benth.,
			Menthinae	*Conradina canescens* (Torr. & A. Gray ex Benth.) A. Gray
	Bignoniaceae	Tecomeae	N/A	*Tecoma stans* (L.) Juss. ex Kunth
		Bignonieae	Bignoniinae	*Bignonia capreolata* L.
	Verbenaceae	Lantaneae	Lantaninae	*Lantana camara* L.
		N/A	N/A	*Callicarpa americana* L.
	Acanthaceae	Justicieae	Justiciinae	*Justicia americana* (L.) Vahl
	Scrophulariaceae	Cheloneae	N/A	*Penstemon digitalis* Nutt. ex Sims
		Cheloneae	N/A	*Penstemon* × *mexicali*
Category 5: Representative species in other orders that are closely related to Lamiales				
Ericales	Cyrillaceae	N/A	N/A	*Cyrilla racemiflora* L.
Asterales	Asteraceae	Rudbeckieae	Rudbeckiinae	*Echinacea purpurea* (L.) Moench
Dipsacales	Caprifoliaceae	N/A	N/A	*Viburnum obovatum* Walter
Category 6: Species that have some relationship to the target weed; the presence of iridoids link the following plants in Gentianales to the Oleaceae (Jensen et al. 2002)				
Gentianales	Apocynaceae	Nerieae	Neriinae	*Nerium oleander* L.
	Rubiaceae	Cephalantheae	Cephalanthinae	*Cephalanthus occidentalis* L.
	Loganiaceae	Gelsemieae	Gelsemiinae	*Gelsemium sempervirens* (L.) W.T. Aiton
Category 7: Cultivated species in other orders				
Solanales	Solanaceae	Lycieae	Lyciinae	*Lycopersicon esculentum* Mill.
	Solanaceae	Capsiceae	Capsicinae	*Capsicum annuum* L.

[a] Family-level taxonomy based on that listed in the USDA Plants Database.

[b] Tribe and Subtribe designations obtained from various sources. If not listed (N/A), then information is either not available or difficult to determine based on the fact that the taxonomy remains unresolved.

separate laboratory trials from August 2009 to June 2012. Each laboratory trial included two to five test species. To control the variation among trials conducted at different times, positive controls (Chinese privet) were always included in each trial. Lace bug gender was determined based on the shape of the terminal sternite (Zhang et al. 2011). Twenty pairs of adults (1 male and 1 female) were caged separately within polyester sleeve cages (25 by 15 cm) placed over randomly selected branches of each test plant, each open end was tied closed with a wire twist-tie. Typically, five plants of each test species were used, with four cages of paired lace bugs on each. However, for some small plants without sufficient branches, additional plants were needed. One week later, 10 branches with cages (henceforth "adult feeding–oviposition cages") containing lace bugs were randomly selected (2 per plant on average) and cut from each test species. Leaves were removed from branches, placed on a flatbed scanner (HP Photosmart C8180 Scanner) and scanned so that damage could be determined at a later date. Feeding damage to leaves was assessed by counting chlorotic spots or points caused by *L. hospita*. Eggs deposited in leaves were counted using a dissecting microscope immediately after scanning. On the same day, lace bug adults in the remaining 10 cages were collected using an aspirator, and sleeve cages (henceforth "combined oviposition–nymphal development cages") were placed back on the same branches to allow potential eggs to develop. This procedure resulted in the same number of lace bugs ovipositing in each of these cages for 1 wk. These remaining caged branches were checked for newly emerged adults which were collected and counted daily for one month; this duration coincides with egg-adult period for this insect (Zhang et al. 2011). Combined oviposition–nymphal development no choice tests provided a chance to investigate the overall performance of lace bugs from host acceptability for oviposition to the ability of their nymphs to develop on each test species.

Nymphal Development No-Choice Tests

To further test whether some plant species supported nymphal development, we selected 14 species that either were oviposited on or had newly emerged adults in previous no-choice tests. Two plants of each test species were used, and sleeve cages were placed onto three branches on each plant (six replicates). Ten first-instar nymphs were placed gently onto the leaf surface in each sleeve cage using a paint brush. *Forestiera acuminata*, *Forestiera godfreyi*, *Forestiera pubescens*, *Forestiera segregata*, *Fraxinus nigra*, *Fraxinus profunda*, *Fraxinus quadrangulata*, and *L. sinense* were tested between June 21 and July 29, 2013. *Chionanthus virginicus*, *Syringa pubescens*, *S. meyeri*, *S. oblata*, *S. vulgaris*, *Jasminum nudiflorum*, *and L. sinense* were tested during August 8 and September 9, 2013. In order to control the variation among trials conducted at different times, Chinese privet was tested in each trial. *Chionanthus pygmaeus* was not tested due to unavailability and two *Ligustrum* spp. were not tested since they are also invasive. After 19 d, the longest time required for a first-instar nymph to develop to an adult (Zhang et al. 2011), branches with sleeves cages attached were cut off and processed to determine the number of *L. hospita* that reached adulthood. The adults recovered from *F. pubescens* and *L. sinense* plants were placed back on corresponding host material for 12 d to ensure they had reached sexual maturity and used in the following multiple generation comparison tests.

Multiple Generation Comparison No-Choice Tests on *F. pubescens* and *L. sinense*

Based on the results of the previous no-choice tests, we selected *F. pubescens* to see if it would support multiple generations of *L. hospita*. Replicates consisted of one pair (male and female) of surviving *L. hospita* adults from *F. pubescens* or *L. sinense* plants placed onto a new corresponding host plant branch of the same species (five replicates per species). Plants were placed individually in Insect Tents (60 by 60 by 60 cm, model BD2120, MegaView Science Co., Ltd, Taichung, Taiwan) where adults were allowed 2 d to oviposit before being removed and placed in to 70% alcohol for later body measurements. F2 generation adults of *L. hospita* were collected and counted 24 d later. Adults from this F2 generation were placed on fresh corresponding host material for 12 d to ensure they reached sexual maturity and used to test their fecundity.

Five sexually mature pairs (male and female) of *L. hospita* from the F2 generation trial were caged on branches (one pair per branch) of the corresponding host plant and left in the cages throughout the trial to compare F2 fecundity. The plants were monitored twice per week for newly hatched nymphs, which were removed, counted, and placed into 70% alcohol. At the end of the trial all F2 generation adults were placed into 70% alcohol for later body measurements which included body length (underside from head to tip of abdomen) and width at widest part of the pronotum.

Multiple-Choice Tests

Most species on which feeding and oviposition occurred in the no-choice tests were tested in multiple-choice tests. In total, 17 plant species from the family Oleaceae were included in the tests. *Chionanthus pygmaeus* is an endangered species and we were unable to acquire more for these for testing, so we used the closely related *C. virginicus*, which can naturally hybridize with it (Elfers 1989), as a surrogate. Because of limited space in the quarantine lab, we divided multiple-choice tests into three trials. In trial one, *F. segregata*, *F. acuminata*, *F. pubescens*, *F. godfreyi*, *S. meyeri*, and *Olea europaea* were tested. In trial two, we tested *Fraxinus pennsylvanica*, *F. caroliniana*, *F. americana*, *F. nigra*, *C. virginicus*, *Ligustrum vulgare*, *and L. japonicum*. In trial three, we tested *Syringa oblata*, *S. vulgaris*, *S. pubescens*, and *Fraxinus latifolia*. *Fraxinus latifolia* was not previously tested in no-choice tests due to availability, but we eventually obtained it so we included it in the final multiple-choice test. *Ligustrum sinense* was included in all trials and each test species was represented by one potted plant. Test plants and control plants were similar in size (about 70–80 cm in height) to provide insects with similar amounts of resource. All plants were randomly placed in a screen cage (213 cm length, 193 cm width, and 244 cm height, with 81 holes per square centimeter) constructed from mosquito netting (Manila Four-Point No-See-Um Bed Canopy/Insect Netting, Nicamaka, Miami, FL). Cages had a slit in one side for access and the bottom edges were taped to the floor to prevent escape. In total, 50 male and 50 female *L. hospita* were released in the middle of the cage but not in contact with any of the test plants. Each test was conducted for 1 wk and consisted of four replicates with new test plants. At the end of each test, leaves of each plant were removed and examined for feeding damage (chlorotic points) and eggs. Leaves with feeding damage were scanned and the damage points were counted. In addition, we made notes on the numbers of adults present on each species at the conclusion of each trial.

Data Analysis

In the adult feeding–oviposition and combined oviposition–nymphal development no-choice tests, data were first tested for normality using the one-sample Kolmogorov–Smirnov test. Except for leaf damage data in trial 1 and trial 5, none of data meet the assumptions of normality, so the nonparametric Kruskal–Wallis one-way analyses of variance (ANOVA) test was performed. Rank data were used in the post hoc test (the Student–Newman–Keuls tests) to perform multiple comparisons among treatments through the ANOVA procedure of SPSS (SPSS Inc. 2001). Leaf damage data in trial 1 and trial 5 met the assumptions of normality and homogeneity of variances. These data were subjected to one-way ANOVA, and treatment means were separated by using the Tukey's HSD test (SPSS Inc. 2001).

In the nymphal development no-choice tests, data did not meet the assumptions of normality and homogeneity of variances; hence so the rank data (PROC RANK; SAS Institute 2000) were analyzed. The number of adults reared from Chinese privet from two trials were similar with the average number (± SE) of adults from the first trial being 7.833 ± 0.703 and from the second trial being 7.833 ± 0.601. There is no significant difference between the number of lace bug adults rearing from Chinese privet from two trial using the independent-sample T test ($\mathrm{df} = 10$; $t = 0.000$; $P = 1.000$; SPSS Inc. 2001). Thus, the number of adults reared from each host plant from two trials were combined, and were analyzed with the general linear models (GLM) procedure of SAS (SAS Institute 2000) with means separated using the Tukey's HSD test (SAS Institute 2000).

In multiple generation comparison no-choice tests, the number of second-generation adults reared and number of nymphs recovered in the third generation from *F. pubescens* and *L. sinense* were compared using the GLM procedure with means separated using the Tukey's HSD test. Third-generation nymph counts were omitted from the analysis if the adults died within the first 5 d (1 *F. pubescens* and 2 *L. sinense*). Body measurements of males and females were combined and analyzed separately using the GLM procedure and Tukey's HSD test for means separation (SAS Institute 2000).

Leaf damage spot and egg number data in multiple-choice test 1, as well as egg number data in multiple-choice test 2 did not meet the assumption of normality, so the nonparametric Kruskal–Wallis one-way ANOVA test was performed. Rank data were used in the post hoc test (the Student–Newman–Keuls tests) to perform multiple comparisons among treatments through the ANOVA procedure of SPSS (SPSS Inc. 2001). Leaf damage spot data in multiple-choice test 2, as well as leaf damage spot and egg number data in multiple-choice test 3 were normally distributed and meet the assumptions of homogeneity of variances, data were subjected to one-way ANOVA, and treatment means were separated by using the Tukey's HSD test (SPSS Inc. 2001). Percentage of lace bug adults present on test plants from all three multiple-choice tests were analyzed using the Chi-square test.

Results

Adult Feeding–Oviposition and Combined Oviposition–Nymphal Development No-Choice Tests

In total, 23 of 45 plant species (51.111%) had no feeding damage, 29 species (64.444%) had no eggs laid on them, and 32 species (71.111%) had no newly emerged adults. Lace bugs fed on 22 plant species, with the feeding damage ranging from as low as 0.396% up to 207.692% of that observed on corresponding Chinese privet control plants in each trial. However, only *F. acuminata*, *F. pubescens*, *S. pubescens*, and *S. oblata* were fed on at the same rate or higher than on Chinese privet. In total, 15 species had eggs deposited on them, ranging from 1.875% to 109.740% of eggs laid on Chinese privet tested at the same time. Among them, *F. acuminata*, *F. segregata*, *F. pubescens*, *L. vulgare*, *S. oblata*, and *S. vulgaris* had as many or more eggs as Chinese privet.

The detailed statistical results are summarized in Table 2. The salient findings are outlined below. In trial 1, *L. hospita* feeding, oviposition, and new adults were significantly different among plant species. Adults fed significantly more on *F. acuminata* and they laid as many eggs on it as they did on Chinese privet. However, fewer lace bugs developed into adults on *F. acuminata* than on Chinese privet. There was significantly less leaf damage on *F. pennsylvanica*; no eggs were observed on *F. pennsylvanica* leaves, but a few eggs were laid since *F. pennsylvanica* had 1.105% as many newly emerged adults as Chinese privet.

In trial 2, no feeding, oviposition or newly emerged adults of *L. hospita* occurred on *O. americanus*. Similarly, no feeding damage or newly emerged adults were detected on *F. godfreyi*. However, no significant difference of number of eggs were laid between *F. godfreyi* and *L. sinense*.

In trial 3, no eggs or newly emerged adults were detected on *O. europaea* and feeding damage was 3.805% of that on *L. sinense*. *Leptoypha hospita* fed less on *F. segregata* than on Chinese privet, with about 73.221% less feeding on it than on *L. sinsense*. Females laid as many eggs on *F. segregata* as on *L. sinense*, but a much lower number developed into adults compared with *L. sinense*.

In trial 4, *Fontanesia fortunei* had no feeding, oviposition, or nymphal development. Adult *L. hospita* fed and oviposited significantly less on *F. nigra*, *C. virginicus*, and *C. pygmaeus* than on Chinese privet. All three species supported some development of *L. hospita* but significantly less than on Chinese privet.

In trial 5, *L. hospita* feeding, oviposition, and development differed depending on the test species. *Forestiera pubescens* supported equal levels of feeding, oviposition, and development as Chinese privet. *Syringa pubescens* had fewer eggs compared to Chinese privet, but similar amounts of feeding damage and newly emerged adults. *Fraxinus americana* was fed on a small amount but no eggs were found on it and no nymphs completed development.

In trial 6, significant differences in feeding, ovipositing, and number of newly emerged adults of lace bugs occurred. *Leptoypha hospita* adults fed and oviposited on *Syringa meyeri* less than they did on Chinese privet, but there was no significant difference in the numbers of newly emerged adults recovered from *S. meyeri* and Chinese privet. *Leptoypha hospita* did not utilize *F.* × *intermedia* or *C. americana*.

In trial 7, *L. hospita* adults fed less on *F. caroliniana* and not at all on *N. oleander* and no eggs or newly emerged adults occurred on either species.

In trial 8, *L. hospita* fed on *L. vulgare* and *L. japonicum* but caused less leaf damage on them than on *L. sinense*. However, females laid as many eggs on *L. vulgare* as they did on Chinese privet, and the number of newly emerged adult from *L. vulgare* was higher than that from *L. sinense*. Fewer eggs were deposited on and very few newly emerged adults occurred on *L. japonicum*. No feeding, oviposition, or newly emerged adults occurred on *B.* × *weyeriana*.

In trial 9, no newly emerged adults occurred on any of the species tested except *L. sinense*. Less feeding damage and fewer eggs were detected on non-Chinese privet test plants. Feeding damage on *F. profunda*, *M. didyma*, and *F. quadrangulata* was 4.552, 1.336, and 0.396% of the feeding damage to Chinese privet, respectively. Likewise, very few eggs were laid on *F. quadrangulata* (14.375%, compared to the control) and *F. profunda* (1.875%, compared to the control).

In trial 10, adults fed significantly less on *S. oblata*, *S. vulgaris*, and *J. nudiflorum* than they did on *L. sinense*. However, *L. hospita* laid equal numbers of eggs on *S. oblata*, *S. vulgaris*, and *L. sinense*, but the number of newly emerged adults was lower on all than on *L. sinense*.

Table 2. Mean ± SE of leaf damage, number of eggs laid, and number of newly emerged adults on various plant species in adult no-choice tests of *L. hospita* conducted during 13 trials ($n = 10$) from August 2009 to April 2012

Trial	Plant species	Leaf damage	Egg number	Newly emerged adult
1	***Ligustrum sinense***	131.3 ± 31.6b	15.4 ± 5.5a	38.0 ± 3.9a
	Fraxinus pennsylvanica	19.9 ± 7.7c	0b	0.4 ± 0.3c
	Forestiera acuminata	272.7 ± 43.0a	16.9 ± 5.1a	10.8 ± 4.0b
		$F_{(2,27)} = 16.55$; $P < 0.001$ (ANOVA)	Chi-Square = 10.159; df = 2; $P = 0.006$ (Kruskal–Wallis H Test)	Chi-Square = 21.332; df = 2; $P < 0.001$ (Kruskal–Wallis H Test)
2	***Ligustrum sinense***	114.5 ± 15.3a	21.8 ± 8.1a	27.7 ± 5.4a
	Forestiera godfreyi	0b	2.40 ± 1.0a	0b
	Osmanthus americanus	0b	0b	0b
		Chi-Square = 27.49; df = 2; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 9.45; df = 2; $P = 0.009$ (Kruskal–Wallis H Test)	Chi-Square = 27.50; df = 2; $P < 0.001$ (Kruskal–Wallis H Test)
3	***Ligustrum sinense***	141.9 ± 19.8a	19.4 ± 4.8a	31.6 ± 6.5a
	Forestiera segregata	38.0 ± 8.2b	9.6 ± 2.5a	2.8 ± 1.0b
	Olea europaea	5.40 ± 3.90c	0b	0c
		Chi-Square = 22.78; df = 2; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 16.58; df = 2; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 19.22; df = 2; $P < 0.001$ (Kruskal–Wallis H Test)
4	***Ligustrum sinense***	145.9 ± 13.0a	41.1 ± 4.1a	49.3 ± 9.5a
	Fraxinus nigra	70.5 ± 12.6b	2.7 ± 1.2c	5.1 ± 1.9b
	Chionanthus virginicus	42.9 ± 9.8b	11.9 ± 2.8b	8.7 ± 2.6b
	Chionanthus pygmaeus	54.3 ± 31.4b	6.8 ± 6.8c	6.0 ± 4.4b
	Fontanesia fortunei	0c	0c	0c
		Chi-Square = 31.95; df = 4; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 34.33; df = 4; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 19.18; df = 4; $P = 0.001$ (Kruskal–Wallis H Test)
5	***Ligustrum sinense***	136.3 ± 13.4a	18.4 ± 2.7a	18.6 ± 5.6a
	Fraxinus americana	15.6 ± 5.7b	0c	0b
	Forestiera pubescens	136.5 ± 21.5a	17.0 ± 2.8a	17.1 ± 3.9a
	Syringa pubescens	143.3 ± 22.7a	5.0 ± 1.9b	5.0 ± 1.7a
		$F_{(3,\ 36)} = 12.79$; $P < 0.001$ (ANOVA)	Chi-Square = 25.29; df = 3; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 15.62; df = 3; $P = 0.001$ (Kruskal–Wallis H Test)
6	***Ligustrum sinense***	90.5 ± 37.1a	22.3 ± 11.39a	20.60 ± 22.48a
	Syringa meyeri	20.6 ± 27.0b	4.8 ± 6.11b	6.00 ± 7.77a
	Forsythia x *intermedia*	0c	0c	0b
	Callicarpa americana	0c	0c	0b
		Chi-Square = 32.39; df = 3; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 31.01; df = 3; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 17.86; df = 3; $P < 0.001$ (Kruskal–Wallis H Test)
7	***Ligustrum sinense***	148.80 ± 14.30a	14.50 ± 2.19a	19.90 ± 3.86a
	Fraxinus caroliniana	66.90 ± 15.92b	0b	0b
	Nerium oleander	0 c	0b	0b
		Chi-Square = 22.19; df = 2; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 27.51; df = 2; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 23.86; df = 2; $P < 0.001$ (Kruskal–Wallis H Test)
8	***Ligustrum sinense***	130.20 ± 8.67a	23.50 ± 3.16a	24.2 ± 10.20b
	Ligustrum vulgare	91.90 ± 10.70b	18.50 ± 3.34a	34.40 ± 5.84a
	Ligustrum japonicum	53.50 ± 11.51c	4.60 ± 1.31b	0.50 ± 0.50c
	Buddleia x *weyeriana*	0d	0c	0c

(continued)

Table 2.. continued

Trial	Plant species	Leaf damage	Egg number	Newly emerged adult
		Chi-Square = 29.80; df = 3; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 30.17; df = 3; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 24.70; df = 3; $P < 0.001$ (Kruskal–Wallis H Test)
9	***Ligustrum sinense***	202.10 ± 24.97a	16.00 ± 3.81a	25.10 ± 5.88a
	Fraxinus profunda	9.20 ± 4.87b	0.30 ± 0.30c	0b
	Fraxinus quadrangulata	0.80 ± 0.80bc	2.30 ± 1.48b	0b
	Monarda didyma	2.70 ± 1.98bc	0c	0b
	Justicia americana	0c	0c	0b
	Tecoma stans	0c	0c	0b
		Chi-Square = 42.69; df = 5; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 26.73; df = 5; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 34.19; df = 5; $P < 0.001$ (Kruskal–Wallis H Test)
10	***Ligustrum sinense***	113.50 ± 28.09a	15.20 ± 4.53a	16.80 ± 5.19a
	Syringa oblata	20.60 ± 5.89b	10.60 ± 3.74ab	3.80 ± 1.78b
	Syringa vulgaris	10.70 ± 3.32b	7.80 ± 2.32ab	2.00 ± 1.48bc
	Jasminum nudiflorum	15.30 ± 6.31b	1.60 ± 0.64bc	0c
	Lycopersicon esculentum	0c	0c	0c
	Capsicum annuum	0c	0c	0c
		Chi-Square = 38.19; df = 5; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 26.73; df = 5; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 34.19; df = 5; $P < 0.001$ (Kruskal–Wallis H Test)
11	***Ligustrum sinense***	155.20 ± 22.36a	9.80 ± 3.15a	8.30 ± 3.73a
	Perovskia atriplicifolia	1.90 ± 1.32b	0b	0b
	Gelsemium sempervirens	0b	0b	0b
	Osmanthus fragrans	0b	0b	0b
	Salvia microphylla	0b	0b	0b
	Lantana camara	0b	0b	0b
		Chi-Square = 51.73; df = 5; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 45.09; df = 5; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 32.66; df = 5; $P < 0.001$ (Kruskal–Wallis H Test)
12	***Ligustrum sinense***	109.60 ± 15.34a	7.70 ± 2.96a	22.10 ± 3.16a
	Penstemon x *mexicali*	2.90 ± 2.05b	0b	0b
	Conradina canescens	0b	0b	0b
	Buddleia cordata ssp. tomentella	0b	0b	0b
	Echinacea purpurea	0b	0b	0b
13	***Ligustrum sinense***	152.80 ± 25.33a	24.00 ± 2.91a	14.4 ± 4.97a
	Penstemon digitalis	0b	0b	0b
	Bignonia capreolata	0b	0b	0b
	Cephalanthus occidentalis	0b	0b	0b
	Cyrilla racemosa	0b	0b	0b
	Viburnum obovatum	0b	0b	0b
		Chi-Square = 52.00; df = 5; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 52.58; df = 5; $P < 0.001$ (Kruskal–Wallis H Test)	Chi-Square = 20.70; df = 5; $P < 0.001$ (Kruskal–Wallis H Test)

Leaf damage is a count of the number of chlorotic feeding points or spots. Data in parenthesis are percentage of damage, oviposition, and successfully developed nymphs on each plant species compared to the corresponding Chinese privet control.

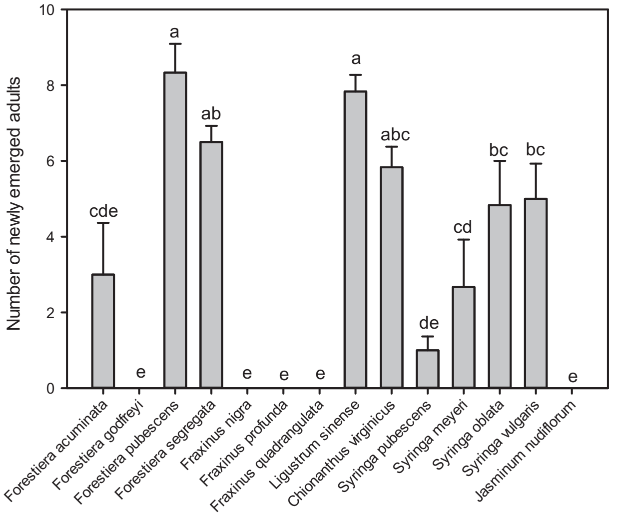

Fig. 1. Number of newly emerged adults on fourteen host plants on which 10 first instars were placed on each plant. All data are presented as mean + SE. Different letters indicate a significant difference among means ($P < 0.05$).

In trials 11, 12, and 13, *Leptoypha hospita* fed a small amount on *P. atriplicifolia* and *P.* × *mexicali*, but no oviposition or newly emerged adults occurred on any of the test species except *L. sinense*.

Nymphal Development No-Choice Tests

The number of newly emerged adults varied depending on host plant ($\chi^2 = 69.654$; df = 13; $P < 0.001$; Fig. 1) . No nymphs survived to adulthood on *F. godfreyi*, *F. quadrangulata*, *F. profunda*, *F. nigra*, and *J. nudiflorum*. *Forestiera pubescens* and the two *L. sinense* treatments (s1 and s2 combined) averaged ∼8 newly emerged adults. *Forestiera segregata* and *C. virginicus* had lower numbers of adults emerge (∼5–6.5 newly emerged adults), but not significantly less than *L. sinense*. *Forestiera acuminata*, *S. oblata*, *S. vulgaris*, *S. meyeri*, and *S. pubescens* had significantly fewer nymphs develop to adulthood (mean 1–3 newly emerged adults) than *L. sinense*.

Multiple Generation Comparison No-Choice Tests on *F. pubescens* and *L. sinense*

Although survival of nymphs to adulthood on these species did not differ in the first generation (Fig. 1), the number of adults recovered after the F2 generation on *F. pubescens* was significantly lower (<1/branch) than on *L. sinense* ($F = 6.149$; df = 3, 18; $P < 0.005$; Fig. 2A). The number of the first instars of the third generation from *L.*

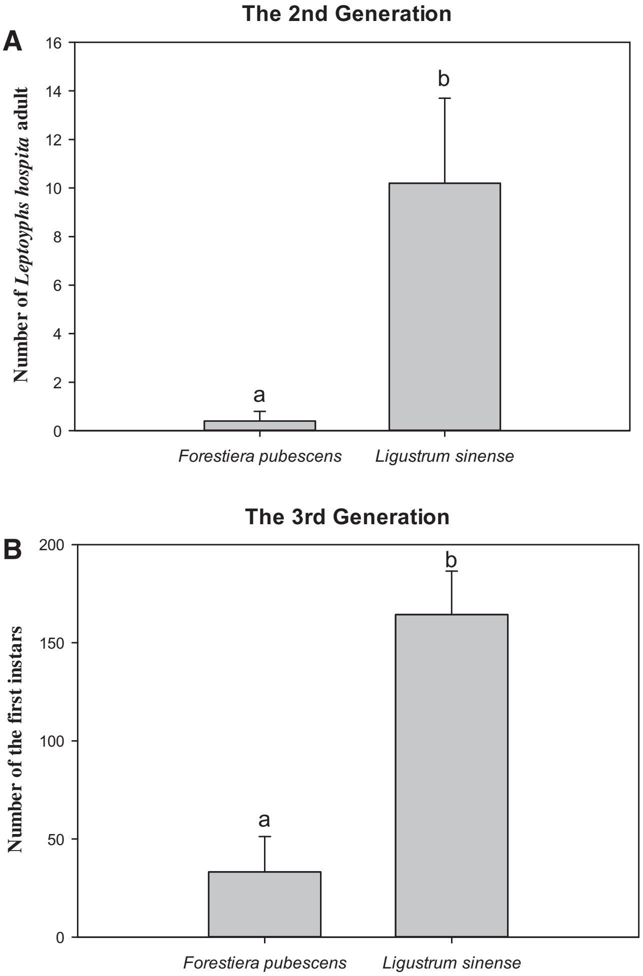

Fig. 2. (**A**) Number of *L. hospita* newly emerged adults reared from *F. pubescens* and *L. sinense* for two generations. (**B**) Fecundity of *L. hospita* measured as the number of third-generation first instar recovered from *F. pubescens* and *L. sinense* after continuous rearing for two generations on the respective hosts. All data are presented as mean + SE. For each parameter, different letters indicate a significant difference among means ($P < 0.05$).

sinense were five times more than those from *F. pubescens* ($F = 21.604$; df = 1, 5; $P = 0.006$; Fig. 2B).

There were significant differences in body length and body width of lace bugs reared from *L. sinense* and *F. pubescens* during two generations (Body length: $F = 10.904$; df = 3, 66; $P < 0.001$. Body width: $F = 63.231$; df = 3, 66; $P < 0.001$; Fig. 3A and B). Lace bugs reared from *F. pubescens* during the second generation were significantly shorter (Fig. 3A), and both first- and second-generation adults were narrower when reared on *F. pubescens* (Fig. 3B).

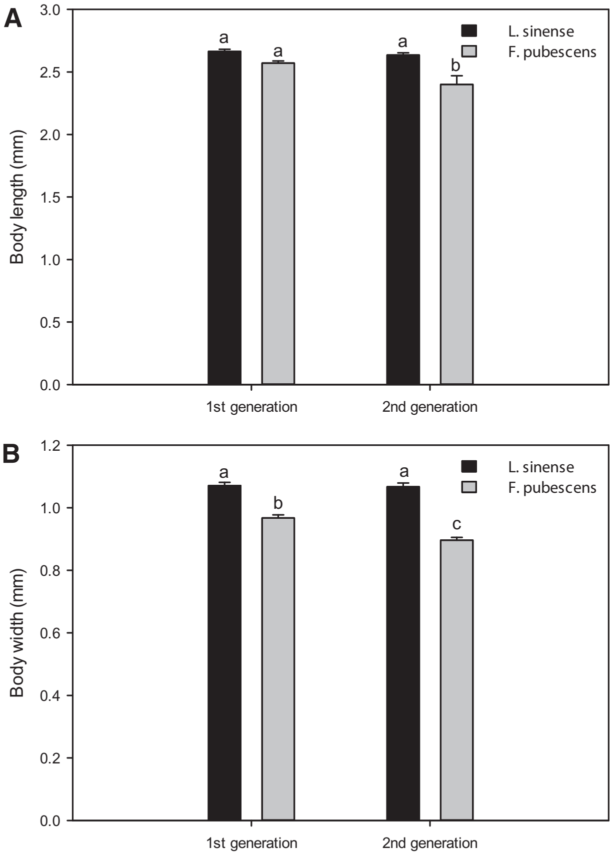

Fig. 3. (**A**) Mean body length and (**B**) mean body width of *L. hospita* adults (males and females data were combined) recovered from *F. pubescens* and *L. sinense* after two generations. All data are presented as mean + SE. Different letters indicate a significant difference among means ($P < 0.05$).

Multiple-Choice Tests

In all multiple-choice trials (Figs. 4–6), leaf damage caused by *L. hospita* (Trial 1: $\chi^2 = 22.897$; df = 6; $P = 0.001$; Fig. 4; Trial 2: $F = 10.888$; df = 7, 24; $P < 0.0001$; Fig. 5A; Trial 3: $F = 28.670$; df = 4, 15; $P < 0.0001$; Fig. 6A) and number of eggs deposited (Trial 1: $\chi^2 = 21.174$; df = 6; $P = 0.002$; Fig. 4; Trial 2: $\chi^2 = 22.862$; df = 7; $P = 0.002$; Fig. 5B; Trial 3: $F = 4.714$; df = 4, 15; $P = 0.012$; Fig. 6A) were significantly different among species. More feeding damage was detected on *L. sinense* than any other species. *Leptoypha hospita* fed very little on other test species in contrast to Chinese privet. *Leptoypha hospita* laid a higher proportion of their eggs on *L. sinense* than other species except the congeneric species *L. vulgare* (Fig. 5B), another invasive *Ligustrum* sp., and three closely related plants *S. vulgaris*, *S. pubescens*, and *S. oblata* (Fig. 6A). The percentage of lace bug adults present on various plants at the end of the study varied (Trial 1: $\chi^2 = 320.811$; df = 6; $P < 0.0001$; Fig. 4; Trial 2: $\chi^2 = 118.110$; df = 7; $P < 0.0001$; Fig. 5B; Trial 3: $\chi^2 = 224.391$; df = 4; $P < 0.0001$; Fig. 6B), but more were found on *L. sinense* than other species except *F. americana* (Fig. 5B), which had similar numbers of adults to *L. vulgare* as well.

Discussion

Adult feeding and oviposition no-choice tests showed that the host range of *L. hospita* was restricted to the tribe Oleeae. Species outside of the Oleeae (Tables 1 and 2) were not attractive to *L. hospita* as evidenced by the lack of feeding, oviposition, and newly emerged adults. Even though *L. hospita* fed sporadically on a few species outside of the tribe Oleeae, such as *M. didyma*, *P. atriplicifolia*, and *J. nudiflorum*, females laid no or very few eggs on them and none yielded adult development. This supports the theory that plants taxonomically closer to the target plant are most likely to be susceptible to relatively host-specific insects (Hinz et al. 2008). Nymphal development no-choice tests further narrowed the host range of *L. hospita* within tribe Oleeae, since several species within that tribe failed to support nymphs developing into adulthood. Among all fundamental host species of *L. hospita*, *L. sinense* was the most suitable

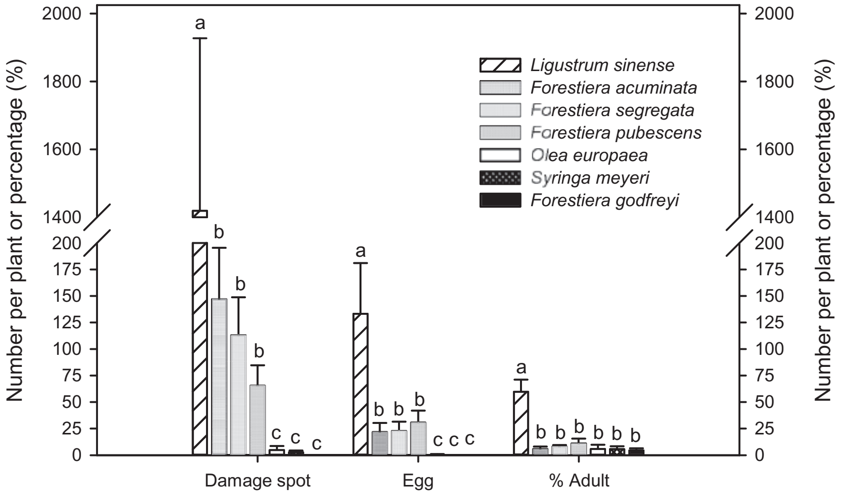

Fig. 4. Leaf damage spots, eggs, and percentage of adults present on each plant species in the multiple-choice feeding and oviposition trial 1. All data are presented as mean + SE. For each parameter, different letters indicate a significant difference among means ($P < 0.05$).

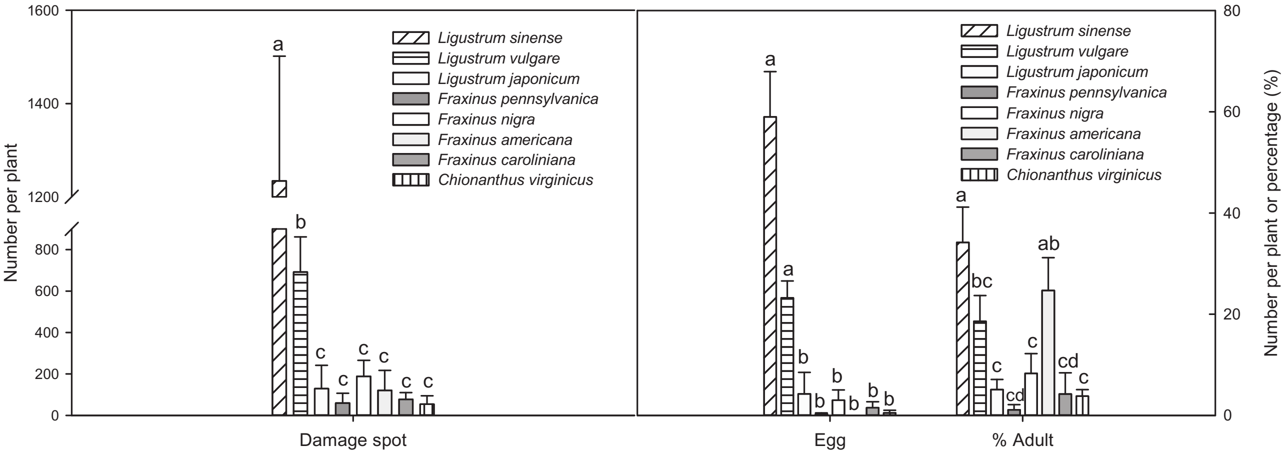

Fig. 5. Leaf damage spots, eggs, and percentage of adults present on each plant species in the multiple-choice feeding and oviposition trial 2. All data are presented as mean + SE. For each parameter, different letters indicate a significant difference among means ($P < 0.05$).

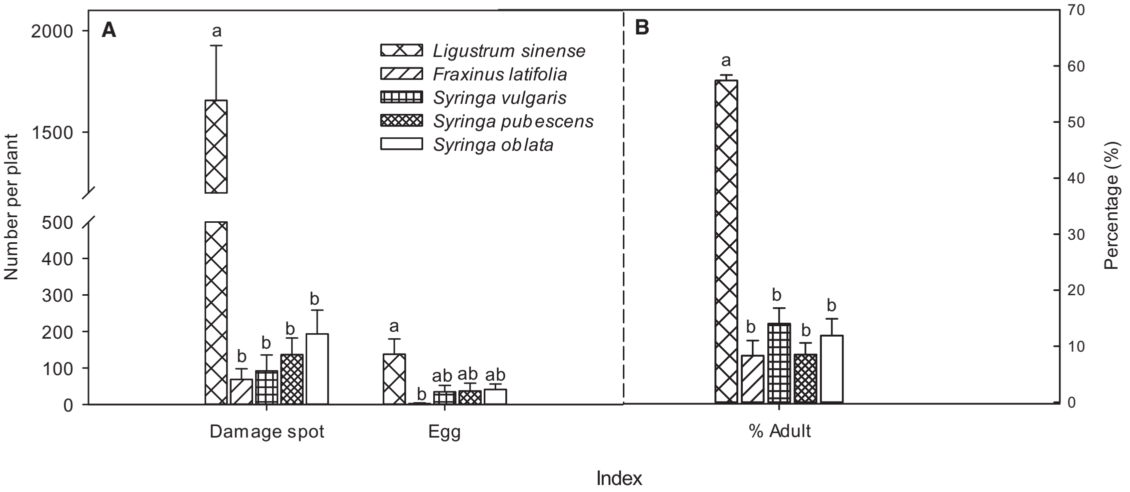

Fig. 6. Leaf damage spots, eggs, and percentage of adults present on each plant species in the multiple-choice feeding and oviposition trial 3. All data are presented as mean + SE. For each parameter, different letters indicate a significant difference among means ($P < 0.05$).

host in multiple generation comparison no-choice tests. In those trials, using the surrogate plant *F. pubescens*, *L. hospita* laid few eggs and survived poorly on *F. pubescens* by the second generation. The low recovery of adults in the second generation on *F. pubescens* may have been due to low fecundity during the 48-h oviposition period, poor survival, or both. In subsequent tests the adults from that generation laid fewer eggs on *F. pubescens* and were smaller in size than those reared on *L. sinense*. Body length and width correlates with overall body mass in most insects (Muhamad et al. 1994), so these results suggest that *F. pubescens* is a poor host resulting in lower body mass and poor fecundity. Since multiple generation comparison no-choice tests showed that *F. pubescens* was not likely to support populations of *L. hospita* long-term under optimal laboratory conditions, it is unlikely they would fare well in a natural setting either.

Due to the constraints of working with *L. hospita* in quarantine, we were unable to study habitat preferences for the lace bug. Despite the difficulty of obtaining these natural history traits that would provide a greater knowledge of the agent, some information suggests the lace bug would do poorly. *Forestiera pubescens* (synonym *F. neomexicana* A. Gray) is distributed from California east to Colorado and Texas. Within that range two states (Oklahoma and Texas) also have Chinese privet (USDA, NRCS. 2013). In addition, *F. pubescens* grows on dry slopes and ridges below 2,000 m (Munz and Keck 1973), while Chinese privet grows in moist riparian habitats. These habitat differences and geographical separation, as well as the lower preference of *L. hospita* for *F. pubescens* in multiple-choice tests suggest that *L. hospita* is unlikely to negatively impact this plant. However, if approved for release, field monitoring of *F. pubescens*, particularly in areas of geographic overlap with *L. sinense*, would be prudent to ensure any unlikely unpredicted nontarget damage by *L. hospita* is detected.

Forestiera acuminata and *F. segregata* occur in the same geographical area currently invaded in North America by Chinese privet. *Forestiera acuminata* occupies the same habitat, the understory shrub layer of riparian forests where *L. sinense* invasion may be slowly displacing its populations (personal observation). *Forestiera segregata* is threatened in the wild by loss of habitat and exotic pest plants (http://georgiawildlife.com/sites/default/files/uploads/wildlife/nongame/pdf/accounts/plants/forestiera_segregata.pdf), including Chinese privet. In multiple-choice tests, *L. hospita* preferred *L. sinense* for oviposition over *F. acuminata* and *F. segregata*. In the absence of the preferred host (Chinese privet) *Forestiera* spp. might be subject to some risk but our results suggest it would be minimal.

Because Chinese privet out competes *Forestiera* spp. (i.e., *Forestiera ligustrina*: Morris et al. 2002) when they co-occur and Chinese privet forms thick monocultures that displace most native plants (Morris et al. 2002, Wilcox and Beck 2007, Hanula et al. 2009, Hudson et al. 2014), *F. acuminata* is in danger of disappearing in native riparian habitats from Chinese privet invasion.

When assessing nontarget affects, not only are native wild plants considered, but ornamental landscape plants as well. *Ligustrum vulgare* was a widely planted ornamental that is also an invasive species with physical characteristics similar to Chinese privet (http://www.invasive.org). *Leptoypha hospita* oviposited and survived on it as well as it did on Chinese privet in no-choice tests although adults did not feed on it in equal numbers. In multiple-choice tests, *L. hospita* preferred feeding on Chinese privet over *L. vulgare*.

Although some genera (*Ligustrum*) have characteristics that assist them to quickly invade natural habitats once they are introduced, other closely related genera apparently do not. Lilacs (*Syringa* spp.) are widely planted ornamentals of Asian origin and phylogenetically related to *Ligustrum* (Wallander and Albert 2000). We found some variation in the acceptability of lilacs as hosts by *L. hospita*. *Syringa meyeri* was fed upon very little in both no-choice and multiple-choice tests. The lace bug laid a few eggs on *S. meyeri* in no-choice tests while they did not lay eggs on it in multiple-choice tests. Likewise, *S. pubescens* experienced feeding in similar amounts to *L. sinense* but lace bugs deposited fewer eggs on it. Conversely, *S. oblata* and *S. vulgaris* had lower feeding damage but similar numbers of eggs laid on them as *L. sinense* in no-choice tests. In multiple-choice tests, *S. vulgaris*, *S. oblata*, and *S. pubescens* had statistically similar numbers of eggs as *L. sinense*. However, results from other researchers show that lilacs might not be suitable hosts for the lace bug over time. In multigeneration rearing trials with *L. hospita* ultilizing four cultivars or species of *Syringa* in New Zealand, *L. hospita* performed poorly and had little or no survival on *Syringa* spp. over the course of two generations (Paynter et al., personal communication, http://www.landcareresearch.co.nz/science/plants-animals-fungi/plants/weeds/biocontrol/approvals/completed/privet/host-range-testing). It appears that lilacs might be at risk for adult feeding and egg deposition; however, they do not appear to be able to sustain lace bug populations over the long term.

No-choice trials often overestimate an insect's host range (van Klinken 2000, Haye et al. 2005, Smith 2007), whereas large-scale choice tests seem to provide a better assessment of an insect's ecological host range. In our multiple-choice tests, *L. hospita* displayed a preference for feeding and ovipositing on *L. sinense* over native North American species. Comparison of *L. hospita* feeding and oviposition on other plant species relative to Chinese privet in no-choice and multiple-choice tests showed that preference for nontarget species was lower in multiple-choice tests. This suggests that if *L. hospita* were present in nature with *L. sinense* and other species in the same tribe, the likelihood of the insect feeding or ovipositing on nontarget species would be low. In addition, recent studies showed that the congeneric native lace bug *Leptoypha mutica* can feed and survive on Chinese privet in no-choice trials, but when given the choice in both lab and field trials it selected the native *F. pennsylvanicus*, the host on which it coevolved. Despite 150 years of exposure to Chinese privet the native lace bug still exhibited fidelity to a familiar host (Kalina 2013). This suggests that *Leptoypha* spp. have narrow ecological host ranges and our results suggest *L. hospita* will be similar.

Our findings indicate that *L. hospita* is restricted to feeding on members of the tribe Oleeae and that when given a choice among members of that tribe it has a strong preference for Chinese privet. In cases where it did develop on a host, our data, along with others showed it does not do well over the course of multiple generations. Based on our findings, we have concluded that the perceptible risk to nontarget plants is low and that *L. hospita* is an excellent candidate for biological control of Chinese privet. Therefore, we have submitted a petition to release this agent to USDA APHIS.

Acknowledgments

We are grateful to Mike Cody for assistance in the quarantine laboratory. We also thank Richard Reardon (USDA Forest Service, Forest Health Technology Enterprise Team) and the Southern Research Station's Insects, Diseases, and Invasive Plants research unit for funding the research.

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