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

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ABSTRACT The biology of *Leptoypha hospita* Drake et Poor (Hemiptera: Tingidae), a potential biological control agent from China for Chinese privet, *Ligustrum sinense* Lour., was studied in quarantine in the United States. Both nymphs and adults feed on Chinese privet mesophyll cells that lead to a bleached appearance of leaves and dieback of branch tips. *L. hospita* has five nymphal instars with the mean duration of the life cycle from egg to new adult being 25 d. Females laid an average of 240 eggs per female and continued laying until death. Adults lived ≈75 d on average. Because it feeds on Chinese privet, has multiple generations per year and a high reproductive rate, *L. hospita* may be a good biological control agent for this invasive plant.

KEY WORDS lace bug, tingid, biocontrol, invasive plant, *Ligustrum sinense*

Invasive plant species, such as Chinese privet, *Ligustrum sinense* Lour. (Oleaceae), are economically and ecologically important. Introduced as an ornamental shrub from Asia in 1852 (Dirr 1983), Chinese privet was naturalized as early as 1933 (Small 1933) and has become one of the most abundant nonnative invasive plants in the southeastern United States (Miller et al. 2008). An aggressive, shade-tolerant evergreen shrub that grows in dense thickets up to 9 m in height (Miller 2003), Chinese privet does well in full sunlight and in the understory of forests, particularly riparian areas. Attributes that enhance its ability to invade include its high growth rate, vegetative reproduction, shade tolerance, and prolific seed production (Langeland and Burkes 1998). It competes with native plants for light and nutrients, reducing native plant diversity and suppressing tree regeneration (Kittell 2001, Morris et al. 2002, Wilcox and Beck 2007, Hanula et al. 2009). It is also a threat to the Schweinitz's sunflower (*Helianthus schweinitzii* Torr. & A. Gray) and Miccosukee gooseberry [*Ribes echinellum* (Coville) Rehder], endangered species occupying the same habitat (Cuda and Zeller 1999, U.S. Fish and Wildlife Service 1994). Once Chinese privet is established in an area, it is difficult to remove. Large-scale control of privet is labor-intensive and requires the use of herbicides (Hanula et al. 2009 and references therein). As is the case with most environmental weeds, biological control presents the most practical control option. Chinese privet is particularly promising because no *Ligustrum* species are native to North America.

A U.S.–China cooperative project on biological control of Chinese privet was initiated in 2005. As many as 170 phytophagous insect species were detected feeding on Chinese privet in China (Zhang et al. 2008). Two insects, *Leptoypha hospita* Drake et Poor (Hemiptera: Tingidae) and *Argopistes tsekooni* Chen (Coleoptera: Chrysomelidae), were selected as the most promising agents for biocontrol based on their recorded host range and the extent of damage they cause. In 2008 and 2009, >100 *L. hospita* were shipped from China to the United States to start a colony that has been maintained in quarantine at the University of Georgia horticultural farm near Watkinsville, GA.

L. hospita is a native of China, Penang Island, and Malaysia (Drake and Ruhoff 1965) where *L. sinense*, *Ligustrum quihoui* Carrière, and *Ligustrum obtusifolium* Siebold & Zucc. were reported as its hosts (Li 2001). During surveys for natural enemies (Zhang et al. 2008) *L. hospita* was found in abundance feeding on leaves of Chinese privet in China. Its feeding resulted in a bleached appearance of leaves and premature defoliation which dramatically reduced the attractiveness of the plants. Despite its damage to this common ornamental shrub in China, little is known about its biology, seasonality, or ecology there. The purpose of this study was to examine its biology under laboratory conditions to aid in future studies on its potential as a biocontrol agent.

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Materials and Methods

L. hospita adults were field collected from *L. sinense* in Huangshan city (29° 24′–30° 31′ N, 117° 12′–118° 53′ E), Anhui province, China, in March 2009. Adults and excised branch tips were packaged and shipped to the United States (USDA–APHIS permit P526P-08-01 107). Upon arrival lace bugs were transferred to potted privet in a quarantine laboratory in Georgia. Plants were covered with white polyester cages (90 cm in height) to prevent insects from escaping. The lace bug colony was maintained in the lab by transferring bugs to new plants as old plants deteriorated. Lace bugs used in our studies all came from this colony maintained at 24–26°C, 50–80% RH, and a photoperiod of 15:9 (L:D) h. Voucher specimens were deposited in the University of Georgia Natural History Museum.

To determine the egg incubation period, 20 male–female pairs of lace bug adults were caged separately within polyester cages (25 by 15 cm) over individual branches. Each day, adult pairs were transferred to fresh branches. Preliminary studies showed that incubation required more than one week so we waited 7 d and cut the branches and inserted each branch into a 30-ml plastic diet cup filled with water. Eggs were inspected every 24 h by using a dissecting microscope until neonates emerged. In total, 75 eggs were monitored for hatching. Ten eggs were measured using a calibrated ocular micrometer mounted within a dissecting microscope to determine egg length, width of the operculum, and width of the egg. The remaining eggs were used to determine developmental time of the various life stages. Of those, 55 were reared successfully to adults.

The number of instars and the duration of each life stage were determined by placing newly emerged neonates individually on a fresh leaf in a 5.4-cm-diameter covered petri dish with moistened filter paper on the bottom. Leaves were replaced and filter paper moistened daily. All petri dishes with nymphs and leaves were placed on the glazed porcelain plates of glass desiccators with water in the bottom. Conditions inside the desiccators were maintained at 70–80% RH and 24–26°C. Nymphs were examined under a dissecting microscope every 24 h to determine whether they had molted to the next instar as indicated by the presence of exuviae. Nymphs were reared and examined as described above until adults emerged. Mortality of each stage was obtained by recording the number of dead insects. Newly emerged adults were collected and sexed to determine sex ratio. We used the ocular micrometer to measure various morphological characteristics of nymphs ($n = 10$ per instar) taken from the colony to determine how to separate instars.

Preoviposition period, fecundity, length of oviposition period, eggs laid over time, and adult longevity were determined for 16 pairs of lace bugs reared to adult in the previous experiment. Pairs of newly emerged adults were placed in petri dishes within the glass desiccators as described above. Adults were examined daily during the preoviposition period; however, once oviposition started leaves were examined every 2 d to count the number of eggs laid. Adult mortality was recorded daily.

Averages are expressed as means ± SE. An independent sample *t*-test was used to compare the body size of female and male *L. hospita* and the lifespan of males and females under controlled conditions.

Results

Eggs. The eggs of *L. hospita* were elongate, curved, sac-like, and yellowish (Fig. 1A). The posterior pole was hemispheric and the cephalic pole was closed by a cup-shaped operculum, which adhered to the head of the neonate nymphs after they emerged. The eggs were inserted so that the operculum was flush with the leaf surface. Eggs averaged 0.45 ± 0.01 mm in length and 0.20 ± 0.01 mm in width, and the mean diameter of the operculum was 0.10 ± 0.00 mm ($N = 10$). Eggs were inserted at an angle in both the dorsal and ventral surfaces of leaves and petioles. The majority of eggs were laid in groups along leaf margins and petioles, although some were laid singly close to the leaf midrib. Eggs could be detected easily because the upper and lower epidermis of the leaf became transparent over the egg and the site of oviposition lost its chlorophyll, exposing the presence of the egg. Eggs hatched in 9–13 d (Table 1).

Nymphs. Nymphs were oblong and flat and newly molted nymphs of each instar were initially pink but turned tawny as the cuticle hardened. The exuviae remained attached to the leaves after each molt. The dorsal surface of the nymphs was covered with short, white granulate setae, similar to that described for *Leptoypha mutica* Say by Mead (1975). There were also short, stout spines in the lateral margins of the thorax and abdomen. *L. hospita* underwent five nymphal molts (Fig. 1B) spending ≈13 d as a nymph before molting to the adult stage. Nymphs had very limited mobility, especially early instars, which stayed in the vicinity of where they hatched. Mortality of immatures was 15.4% with most occurring during the first and second instars (Table 1). After hatching, nymphs began piercing the epidermis of leaves with their stylets and sucking out the mesophyll tissues, which usually resulted in a chlorotic, bleached appearance of the leaves. Of the various body characters of nymphs (Table 2), width across the eyes was the most stable parameter to separate instars. Wing bud development began in the fourth instar and they extended to the second abdominal segment. In the fifth instar wing buds reached the fourth abdominal segment. The mean duration of the life cycle of *L. hospita* from egg to adult emergence was 24.6 ± 0.18 d.

Adults. The sex ratio of female to male was 1.5:1. Adults were narrow-elongate, grayish to brownish gray and mottled with black (Fig. 1C). Sizes of various female and male body characteristics are listed in Table 2. Females were significantly longer ($t = 7.318$, df = 18, $P < 0.001$) and wider ($t = 8.641$, df = 18, $P < 0.001$) than males (Table 2; Fig. 1C). Males and females are easily separated by the shape

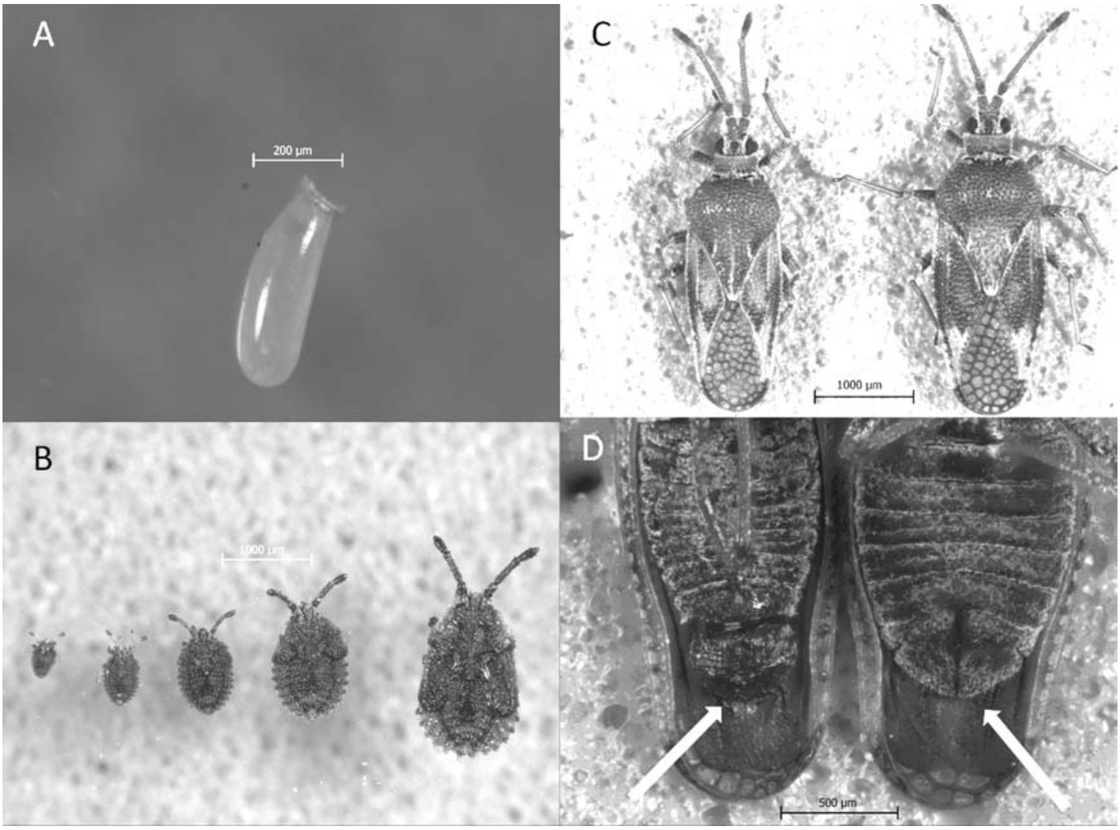

Fig. 1. Photographs of life stages and abdominal sternites of *L. hospita.* (A) Egg. (B) Five nymphal instars. (C) Dorsal view of a male (left) and a female (right). (D) Ventral view of the male with a rounded genital capsule (left) and the female with an ovipositor (right).

of their terminal sternites. In the males, the end of the sternite is a rounded convex genital capsule (Fig. 1D), whereas the female's was smooth with a visible ovipositor (Fig. 1D).

The preoviposition period was 11.6 ± 0.45 d (mean ± SE; range, 9–17 d). After that, egg deposition by females increased sharply peaking 35–45 d after they emerged. Approximately 46 eggs were laid per female during the peak 10-d period. They continued laying eggs until death (Fig. 2). On average, *L. hospita* females produced 240.0 ± 45.81 eggs per female (range, 39–575 eggs). Adult males survived an average of 60.1 ± 8.40 d (range, 21–127 d), whereas females lived an average of 79.9 ± 10.79 d (range, 27–150 d). Although females lived slightly longer than males, female longevity was not significantly longer than males ($t = -1.45$, df = 30, $P = 0.159$).

Discussion

These studies on the basic biology of *L. hospita* are important to aid in culturing this insect in the laboratory and for potential mass rearing, and to provide essential information for designing further host specificity tests. Of the *Leptoypha* species native to North America, *L. mutica* and *Leptoypha costata* Parshley have been studied in some detail. *L. mutica* has at least two generations per year in New Jersey, and like most

Table 1. Duration (in days) and mortality of immature stages of *L. hospita* under laboratory conditions ($N = 55$)

Stage	Range	Mean ± SE	Cumulative mean age	% mortality (no. dead/total no.)
Egg	9–13	11.2 ± 0.13	11.36	1.5 (1/65)
Nymph				
First instar	1–4	2.7 ± 0.08	14.07	7.8 (5/64)
Second instar	1–3	2.0 ± 0.06	16.03	6.8 (4/59)
Third instar	2–3	2.2 ± 0.05	18.19	0 (0/55)
Fourth instar	2–4	2.8 ± 0.06	20.99	0 (0/55)
Fifth instar	3–5	3.8 ± 0.08	24.77	0 (0/55)

Table 2. Measurements (mean ± SE) of *L. hospita* nymphs and adults ($N = 10$)

	Nymph					Adult	
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Female	Male
Body length (mm)	0.58 ± 0.01	0.81 ± 0.00	1.05 ± 0.01	1.41 ± 0.02	1.99 ± 0.02	3.05 ± 0.02	2.82 ± 0.03
Body width (mm)	0.30 ± 0.10	0.43 ± 0.01	0.67 ± 0.00	0.93 ± 0.01	1.13 ± 0.02	1.11 ± 0.01	0.96 ± 0.01
Width across eye (mm)	0.18 ± 0.06	0.24 ± 0.00	0.32 ± 0.00	0.40 ± 0.00	0.47 ± 0.00	0.44 ± 0.00	0.41 ± 0.01
Stylet length (mm)	0.32 ± 0.10	0.36 ± 0.00	0.51 ± 0.00	0.59 ± 0.00	0.73 ± 0.00	0.80 ± 0.01	0.73 ± 0.01
Antenna length (mm)							
First segment	0.02 ± 0.01	0.02 ± 0.00	0.04 ± 0.00	0.07 ± 0.00	0.10 ± 0.00	0.16 ± 0.00	0.16 ± 0.00
Second segment	0.02 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.06 ± 0.00	0.10 ± 0.00	0.12 ± 0.00	0.12 ± 0.00
Third segment	0.08 ± 0.02	0.10 ± 0.00	0.14 ± 0.00	0.21 ± 0.00	0.38 ± 0.00	0.62 ± 0.01	0.66 ± 0.01
Fourth segment	0.09 ± 0.03	0.12 ± 0.00	0.14 ± 0.00	0.18 ± 0.00	0.26 ± 0.00	0.26 ± 0.01	0.28 ± 0.00

other *Leptoypha* species, they overwinter as adults probably in the leaf litter (Mead 1975, McAtee 1917) or in bark crevices. Adult *L. mutica* first appear on host foliage in early April and can remain as late as 3 November in Florida (Mead 1975). In Missouri *L. costata* had three generations per year but adults did not appear on host foliage until 20 May (Sheeley and Yonke 1977).

Immature development of *L. hospita* took 25 d, similar to that of *Carvalhotingis visenda* Drake & Hambleton and *Gargaphia decoris* Drake reported as 29 and 26–33 d, respectively (Olckers 2000, Dhileepan et al. 2010). However, *L. hospita* immature developmental time was ≈12 d shorter than that of *L. costata* reared under similar conditions (Sheeley and Yonke 1977). *L. hospita* adults lived an average of 75 d, which is much longer than *C. visenda* (24–48 d; Dhileepan et al. 2010), *Corythucha gossypii* F. (25 d; López et al. 1982), and *G. decoris* (55 d; Olckers 2000). No information is available on adult longevity of North American *Leptoypha* spp. Fecundity of *L. hospita* was high with an average female laying 240 eggs, almost 3 times as many as *C. visenda* (82 eggs per female) and 1.5 times as many as *G. decoris* (163 eggs per female) (Olckers 2000, Dhileepan et al. 2010). The short period of immature development, long adult lifespan, and high fecundity contributed to quick colony growth under laboratory conditions. Olckers and Borea (2009) demonstrated excised leaves significantly and adversely affected nymph survival rate and development period of *G. decoris.* We also fed immature insects excised leaves, which are a lower quality food source than intact plants and thus might have extended development time and reduced fecundity from what would be observed in the field. If that is the case, then *L. hospita* may develop faster on intact plants. We observed 15% mortality in our trials, all occurring during the first and second instar stages. Disturbance of nymphs by changing leaves in the petri dish, potentially lower nutritional quality of excised leaves, and vulnerability of earlier instars nymphs may all have contributed to this mortality. However, our results are similar to Sheely and Yonke (1977) who also reported high mortality among early instar nymphs of *L. costata.*

Aggregation is an important life history strategy of tingids. For example, Sajap and Peng (2010) reported

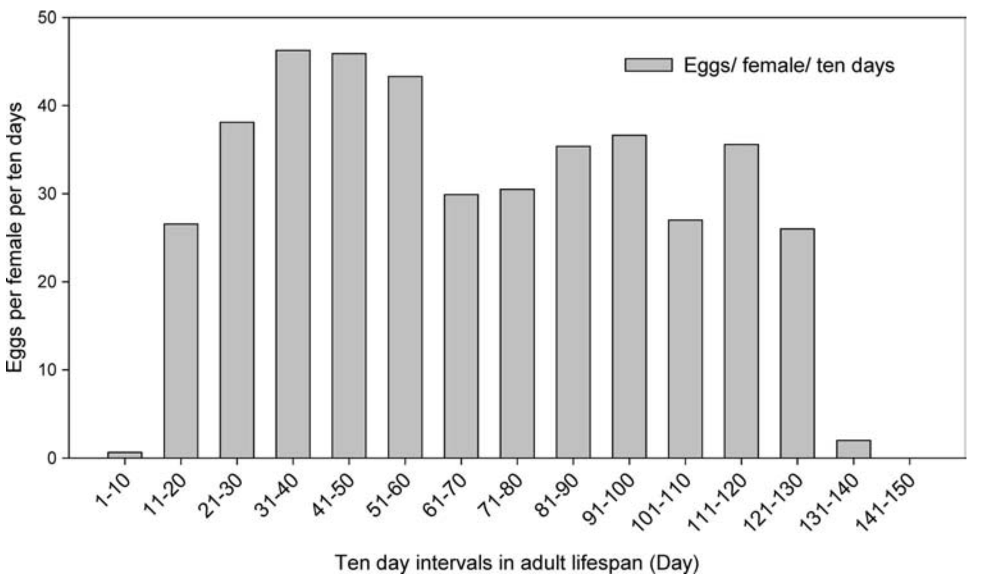

Fig. 2. Number of eggs deposited by *L. hospita* females ($N = 16$) at 10-d intervals under laboratory conditions of 65 ± 10% RH, 25 ± 1°C, and a photoperiod of 15:9 (L:D) h.

that nymphs and adults of *Cochlochila bullita* Stål fed gregariously on the leaves. *G. decoris*, *Telenonemia scrupulosa* Stål, and *Leptobyrsa decora* Drake are also gregarious feeders (Olckers 2000, Harley and Kassulke 1971). However, *L. hospita* behavior was similar to *Teleonemia elata* Drake and *Teleonemia harleyi* Froeschner (Harley and Kassulke 1971) in that we saw little evidence of aggregation in this species under laboratory conditions.

Neal and Schaefer (2000) reviewed the biology and life history of economically important lace bugs. Most lace bugs overwinter as adults, for example, *Corythucha ciliate* Say, *Corythucha cydoniae* Fitch, *Stephanitis pyri* F., and *Urentius hystricellus* Richter. We were not able to determine the overwintering stage of *L. hospita* in our study under quarantine condition. However, during field surveys in China only adults were present on foliage in late fall and again in early spring, suggesting they overwinter (Y.Z.Z., unpublished data), which is consistent with other *Leptoypha* spp. (Mead 1975, Sheeley and Yonke 1977).

Most tingids in temperate zones are univoltine or bivoltine (Drake and Ruhoff 1965). The North American native *Leptoypha elliptica* McAtee was observed to have two generations (Wheeler 2002) in Tennessee and South Carolina. *L. mutica* and *L. costata* were reported to be bivoltine or trivoltine (Dickerson and Weiss 1916, Sheeley and Yonke 1977). We are uncertain how many generations *L. hospita* has per year, but in China it could be found in the field from late March to October, with both nymphs and adults present during most of this period (Y.Z.Z., unpublished data). Under laboratory conditions it has overlapping generations throughout the year on potted *L. sinense.*

It is important to know the duration of the preovipostion period as well as when peak oviposition occurs for host specificity testing. In both no-choice and multiple-choice oviposition trials females at peak egg production are needed. In addition, because adults have a relatively long lifespan, the number of eggs laid per day may decline with age which might affect the results of oviposition tests. In our studies, *L. hospita* has a relatively long preoviposition period of 11.6 d. In comparison, *Corythauma ayyari* Drake had a preovposition period of only 3.7 d (Nair and Nair 1974). Once female *L. hospita* started laying eggs, they laid a relatively constant number each day throughout their lives (Fig. 2). Therefore, females >12 d old should be suitable for host testing.

Lace bugs have been used successfully in biological control of invasive plants worldwide. So far, seven species of tingids have been released as biological control agents against three weeds (Conrad and Dhileepan 2007). *T. scrupulosa* is one of the natural enemies established to control *Lantana camara* L. (Verbenaceae) worldwide (Julien and Griffiths 1998), and it is considered one of the three most successful agents on this plant (Cilliers 1983, Baars and Neser 1999, Baars and Heystek 2003). *L. decora* and *T. harleyi* are two additional tingids established on *L. camara* with varying success (Day et al. 2003). *G. decoris* is thus far the only agent released for the invasive tree *Solanum mauritianum* Scop. (Solanaceae). It has become established in South Africa with reports of severe damage to *S. mauritianum* at a few field sites. Based on its excellent performance in South Africa, an application for permission to release *G. decoris* in New Zealand will be submitted to the regulatory authority (Olckers and Borea 2009).

One major factor contributing to the success of tingids as biological control agents is their biology (Pecora et al. 1992, Olckers 2000). Our study indicates that *L. hospita* has potential as a biocontrol agent as well, based on its biological attributes that include 1) both nymphs and adults feed on the target plant; 2) a short immature developmental period of ≈25 d; 3) multiple overlapping generations observed in the rearing colony in quarantine, and presumably in the field; and 4) long-lived adults with high fecundity.

Some failures of biological control agents to establish or be effective in their introduced range were attributed to the effects of natural enemies (Goeden and Louda 1976, Crawley 1989). No information on the natural enemies of *L. hospita* is available. However, in the process of rearing lace bugs in the laboratory, a minute pirate bug (*Orius* sp.; Hemiptera: Anthocoridae) was found feeding on nymphs. Some natural enemies of lace bugs summarized by Neal and Schaefer (2000) also could potentially migrate to *L. hospita* and the presence of other native *Leptoypha* species in the southeastern United States may increase the probability of that happening.

Host specificity is the most important aspect for potential biological control agents. According to Drake and Ruhoff (1965) tingid species are highly specialized. The current recorded hosts of *L. hospita* are restricted to the genus *Ligustrum* in China (Li 2001). In the United States, there are nine *Leptoypha* spp. that are mainly restricted to plants in the Oleaceae (i.e., *Chionanthus*, *Forestiera*, and *Fraxinus;* http://bugguide.net/node/view/36807). For example, the native lace bug *L. ilicis* and its co-occurring syntopic *L. elliptica* occur on *Forestiera ligustrina* (Michx.) Poir. (Oleaceae) and *Forestiera acuminata* (Michx.) Poir. (Oleaceae) (Wheeler 2002). *L. mutica* was recorded feeding on *Chionanthus virginicus* L. (Oleaceae) and *Fraxinus* spp. (Mead 1975), and even occasionally on *L. sinense* in very low numbers (Y.Z.Z., unpublished data). *L. hospita* is currently being tested in no-choice and multiple-choice feeding and oviposition host specificity trials in quarantine to determine whether it is equally host specific. The biological information obtained from this study is being used to aid in designing host range tests of *L. hospita* in quarantine.

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