

POTENTIAL OF A NATIVE LACE BUG (*LEPTOYPHA MUTICA*) FOR BIOLOGICAL
CONTROL OF CHINESE PRIVET (*LIGUSTRUM SINENSE*)

By

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(Under the Direction of S. Kristine Braman and James L. Hanula)

Abstract

This work was done to better understand the lace bug *Leptoypha mutica* Say (Hemiptera: Tingidae) and its potential for biological control of Chinese privet, *Ligustrum sinense* Lour (Oleaceae). *L. mutica* host utilization of Chinese privet was confirmed in a no choice test. In choice tests with plant species Chinese privet, swamp privet (*Foresteria acuminata* Michx), and green ash (*Fraxinus pennsylvanica* Marsh) *L. mutica* preferred green ash. The duration of development of *L. mutica* on Chinese privet from egg to adult ranged from 24.4 to 57.1 days at the temperatures: 20°C, 24°C, 28°C, and 32°C. Estimated threshold temperatures (T_o) and calculated thermal unit requirements (K) for development of egg, nymphal, and complete development were 11.0°C, 9.9°C, 10.5°C and 211.9, 326.8, 527.4 degree-days (DD). The only suspected specific *Leptoypha* spp. natural enemy found was a mymarid wasp, but was not concluded to be limiting factor for *L. hospita*'s success.

INDEX WORDS: Chinese privet, *Ligustrum sinense*, *Leptoypha mutica*, biological control, invasive, biology, host study, no choice study, choice study, natural enemy complex.

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DEDICATION

I dedicate this work to the good Lord who opened a door for every closed one, and so carefully put so many integral people and opportunities in my life to get me to this point.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

In the southeastern United States, Chinese privet (Oleaceae), *Ligustrum sinense* Lour has become a pervasive, exotic plant, in part due to its aggressive adaptability to a wide range of environments and habitats. It tolerates a range of light, temperature and soil conditions, despite its tropical origins (Brown and Pezeshki 2000). Surveys show that of the ≈ 7.1 million hectares where the privet occurs, it densely covers ≈ 1 million hectares (Miller et al. 2008; Rudis et al. 2006). Chinese privet is listed as a category 1 invasive species according to The Florida Exotic Pest Council, and according to the Nature Conservancy is a major concern for forest ecosystems in Georgia (FLEPPC 1996; The Nature Conservancy 2004).

It is apparent that invasive plants can considerably decrease native plant biodiversity by competing for water and other nutrients (Morris et al. 2002). Chinese privet also competes for light by producing a shade cover over natural vegetation on the forest understory (USDI Fish and Wildlife Service 1992; Merriam & Feil 2002). Recent studies by Hanula et al. (2009) demonstrated that Chinese privet can reduce forest overstory plants. When dense Chinese privet in the forest understory was removed, recovery of overstory was observed. Invertebrates associated with plant communities can be affected by invasive shrubs (Biesmeijer et al. 2006; Crisp et al. 1998). Studies have shown that three times as many bee and butterfly species occur where Chinese privet had been removed five years previously and 5 – 10 times more individuals were trapped in

those areas compared to heavily infested forest (Hudson 2013). Chinese privet can potentially cause harm to humans and animals if its fruits are ingested. Toxic effects can cause nausea, headache, vomiting, abdominal pain, low blood pressure and diarrhea if ingested. The pollen can also potentially cause allergies such as hay fever and asthma (Kerr and Kelch, 1999).

Chinese privet is a growing threat with impending consequences and no easy solution in sight. One possibility is the use of lace bugs (Tingidae) as a biological control agent. Tingids may be useful for biological control because of their host specificity (Drake and Ruhoff 1965). However, lace bug host specificity sometimes extends to other members of a genus or plant; for example the azalea lace bug, *Stephanitis pyrioides* Scott feeds on plants in the family Ericaceae, but there are limited reports of *S. pyrioides* extending feeding to different plant families (Nair and Braman 2012). Surveys of natural enemies conducted in China concluded *Leptoypha hospita* Drake et Poor (Hemiptera: Tingidae) functioned as a biological control agent (Zhang et al. 2008) of Chinese privet. In China, it is the most common insect feeding on it and causes dieback of branch tips. The biology of *L. hospita* was studied in quarantine in the United States (Zhang et al. 2011). *L. hospita*'s promising potential as a control agent introduced the idea of further examination of native, congeneric lace bug species, *Leptoypha mutica* Say, currently utilizing swamp privet (Oleaceae), *Foresteria acuminata* Michx (Mead 1975). Swamp privet is a deciduous shrub or tree reaching up to 10 m in height and is similar to the Chinese privet in habitat (Krüssmann 1986, Johnson and Hoagland 1999). It is found in swamps, wetlands, forest bottoms, around ponds and lakes in the Southeastern United States (Johnson and Hoagland 1999). It ranges from South Carolina to Florida and

westward all the way into Texas, Oklahoma and Kansas. It extends northward as far up as Indiana, Missouri, and Illinois (Krüssmann 1986). Other hosts of *L. mutica* include fringe trees, *Chionanthus virginicus* L., and green ash, *Fraxinus pennsylvanica* Marsh, both in the family Oleaceae (Drake 1918). The similarities between these Oleaceae plant species suggest potential for the native *Leptoypha* to utilize the invasive Chinese privet. By studying the regulating factors, host plant preference, biology of the lace bug, and natural enemy complexes associated with Chinese privet, answers may be obtained on suppressing the invasive using insect biocontrol.

Background on Chinese Privet. Chinese privet, *Ligustrum sinense*, is a perennial, semi-evergreen shrub growing up to 10 m in height belonging to the Oleaceae family. (Faulkner et al. 1989; Stone 1997). It is natively found in China, Vietnam, and Laos (The Nature Conservancy 2004; Wu & Raven 2003), and is found in valleys, forest, along rivers, thickets, woods, and canyons inhabiting elevations from 200 to 2600 m (Wu & Raven 2003; Qui et. al. 1992). Chinese privet is grown as an ornamental shrub with its berries used for brewing, and its oils extracted from its seeds for soaps. In these areas, it has never been reported as harmful to agricultural nor forest settings (Qui et al. 1992).

Chinese privet was brought to the United States in 1852 with intentions of being used as an ornamental shrub (Coates 1965; Dirr 1990). The species escaped cultivation and by 1932 inhabited much of the Southeastern United States (Small 1933). Currently, the species is found ranging from Texas to Florida, all the way north to the New England states. It has also been recorded in Puerto Rico (USDA- NRCS 2002), Argentina (Montaldo 1993), and Australia (Burrows and Kohen 1986). It is commonly found alongside wood lines, stream banks, forest borders, fences, and forest areas that are often

flooded. These areas are similar to those in China in which the plant natively thrives (Langeland and Burkes 1998).

Chinese privet has leafy branches nearly year round and produces clusters of small, white flowers attractive to various bees from April to June. The fruits produced are drupe and ovoid in shape and occur from October to February. The fruit begins as a green color and matures to purple/ black with one to four seeds inside. Chinese privet becomes sexually mature around four years and each privet plant can produce up to 3000 drupes per stem. A large, mature Chinese privet can produce up to a million seeds per year. The seeds have forceful reproductive biology and almost 100% of privet seeds are viable (Mowatt 1998). The drupes are eaten by birds and a variety of mammals where they then spread the seeds to various locations and habitats. (Mazia et al. 1996; Stromayer 1996). The seeds can then germinate in temperatures ranging from 15° C to 25° C (Mowatt 1998).

Information on Tingidae. Lace bugs belong to the order Hemiptera and family Tingidae. They get their name by the lacework appearance of their upper bodies. They are small insects, normally no longer than 5 mm. Lace bugs are broadly dispersed throughout the world with over 2,000 species and around 250 genera (Schuh 1995). The life cycle is similar among species with eggs being found in the leaf tissue. Lace bugs have 4 to 5 nymphal instars and take 5 to 7 weeks to complete their life cycle. Both the adults and nymphs of this family are phytophagous and are normally host restricted. They typically feed and develop on plants of one genus or several related genera. Often they are found on woody plants and feed on the underside of the leaves. By feeding on the chlorophyll of the mesophyll tissue they produce a bleached, chlorotic appearance on the

leaf's upper surface. Black excrement spots or frass are present on the upper and lower surface of the leaf. With heavy infestation, there is graying and chlorosis of the upper leaf surface, premature dropping of leaves and possible death to the plant (Wheeler 2002).

Potential for biological control: *Leptoypha mutica*. F.W. Mead from the Florida Department of Agriculture and Consumer Services first described *Leptoypha mutica* (Mead 1975). The lace bug adult is described as elongate with a length of 2.7 – 3.0 mm and width of 0.9 – 1.0 mm. In general, females are slightly longer and wider than males. They range in color from reddish brown to a light or dark brown. The forewings have a variable blackish spot at the middle and most of the time have black edges and some black veins on the membrane. The pronotum is humped with only one dorsal ridge, but does not have an inflated hood or paranota. The nymphs are characterized by being oblong and flat. Short white setae cover the dorsal surface. Short spines occupy the lateral margins of the thorax and abdomen. The head has a pair of spines, and darkened wing pads are present on the last two instars. The mature nymph is around 1.8 mm long and 1.2 mm wide. The eggs of *Leptoypha mutica*'s eggs are around 0.3 mm, smooth, yellowish and flaskshaped. They are mostly found inserted along the ventral midrib in small clusters. The lace bugs cause damage by feeding on the mesophyll tissue of the leaves browning and wilting them. Severe infestation can cause death to the plant. They are normally found feeding on the basal surface of the leaf, but in some cases where the leaves are not exposed to direct sunlight, they will also feed on the upper surface (Mead 1975).

L. mutica has been reported to be distributed from Maine, Quebec, Ontario, west to North Dakota, and south to Florida and Texas, as well as, Socorro Island, Mexico. In

Florida, the lace bug adults have been collected from fringe trees from April 24 – November 3. It is indicated that they have two broods each year and overwinter as adults (Mead 1975; Barber and Weiss 1922). According to Drake (1918), specimens were collected off of *Forestiera acuminata*, swamp privet, in Texas. This is the only lace bug that has been found to breed on fringe trees, but also is hosted by ash trees.

Similar native lace bug species: *Leptoypha ilicis* and *Leptoypha elliptica*.

Leptoypha elliptica Mcatee adults are 2.8 to 3 mm long, broadly elliptical, widest at the middle of the forewings. The forewings are broadly rounded at their tips. *Leptoypha ilicis* Drake is more elongate, the body nearly subparallel, and about 2.2 mm long. Both occur on host plants upland swamp privet, *Forestiera ligustrina*, and eastern swamp privet, *Forestiera acuminata*. All known distributions of these lace bugs fall within the locations of their principal hosts. Not much is known about them, but they are known to be mesophyll feeders found on lower leaf surfaces that cause bleaching of the leaves of affected plants. Similar to other *Leptoypha* species they are assumed to overwinter as adults and become active in the early spring. A study showed late instars of *L. elliptica* in mid-April in Tennessee and South Carolina and collection of fifth instars of *L. elliptica* in Tennessee in late May and collections of *L. ilicis* in Georgia in early July. These collections suggest a second brood (Wheeler 2002).

Similar exotic lace bug species: *Leptoypha hospita*. *Leptoypha hospita* has been suggested as a promising biological control agent of *L. sinense* because of its narrow host range and extent of damage caused on the plant evaluated in a natural enemies survey. In China, *L. hospita* was found in abundance on Chinese privet and in turn resulted in bleaching of the leaves and premature dropping of leaves (Zhang et al. 2008). This lace

bug is a native of China, Penang Island, and Malaysia (Drake and Ruhoff 1965) where it feeds on *L. sinense*, *L. quihoui* Carrière and *L. obtusifolium* Sieb & Zucc (Li 2001). To examine the biology of the lace bug, it was brought to the United States and studied in a quarantine lab at a University of Georgia horticultural farm near Watkinsville, Georgia.

Results of the studies indicate that *L. hospita* eggs are elongate, curved, sac-like, and yellowish. On average the eggs are 0.45 ± 0.01 mm in length and 0.20 ± 0.01 mm in width. The eggs are inserted flush with the leaf's surface. They are most often found along the leaf margins and petioles and some in the midrib on both the dorsal and ventral surface of the leaves. The eggs hatch within 9 – 13 days. The hatched nymphs are oblong and flat. Once each instar molts, they are initially pink but turn yellow-brown as the cuticle hardens. The dorsal surface of the nymph is covered with short white setae and have short spines in the lateral margins of the thorax and abdomen. There are a total of five nymphal molts lasting 13 days until the nymph reaches adult. The nymphs feed on the mesophyll tissue causing chlorotic bleaching. On average it takes 24.6 ± 0.18 days for *L. hospita* to cycle from eggs to adults. Adults are narrow – elongate with a grayish, brown, and eventually molt to black color. Females are larger than males in length and width. The sex ratio of female to male is 1.5:1 (Zhang 2011).

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CHAPTER 2

HOST UTILIZATION OF CHINESE PRIVET AND
HOST CHOICE BY *LEPTOYPHA MUTICA*

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ABSTRACT Lace bugs (Hemiptera: Tingidae) have been evaluated for potential biocontrol of pervasive, exotic Chinese privet (*Ligustrum sinense* Lour). This study was conducted to determine lace bug *Leptoypha mutica*'s ability to utilize Chinese privet and determine its preferred host plant. A no choice test was done to determine the lace bug's acceptance and utilization of three plant hosts: fringe tree (*Chionanthus virginicus* L.), swamp privet (*Foresteria acuminata* Michx), and Chinese privet based on frass production, oviposition, and survival of lace bugs. Choice tests in the lab and field evaluated lace bug preference among swamp privet, Chinese privet, and green ash (*Fraxinus pennsylvanica* Marsh). All plant hosts supported lace bugs, but there was less egg production on Chinese privet. Green ash was the most preferred, while Chinese and swamp privet were equally preferred. These results were consistent in the field choice test. Chinese privet is a suitable host for *L. mutica*, but may not be an optimal host preventing the lace bugs establishment.

KEY WORDS *Leptoypha mutica*, fringe tree lace bug, *Ligustrum sinense*, Chinese privet, invasive, biological control, host study, no choice study, choice study.

In almost all of the southeastern United States, Chinese privet (Oleaceae), *Ligustrum sinense* Lour, has become a widespread, exotic plant, found on ≈ 7.1 million hectares and densely covers ≈ 1 million hectares of that area (Miller et al. 2008; Rudis et al. 2006). Chinese privet's success can be attributed to its aggressive adaptability to a wide range of environments and habitats (Brown and Pezeshki 2000). In addition, Chinese privet is characterized by a high growth rate, vegetative reproduction and prolific seed production (Langeland and Burkes 1998). Once it is established, the privet becomes very difficult to remove, and large-scale control requires extensive labor and the use of herbicides (Hanula et al. 2009). The Chinese privet causes problems by depleting native plant biodiversity, decreasing invertebrate communities, and having toxicity effects on humans and animals (Morris et al. 2002; Biesmeijer et al. 2006; Crisp et al. 1998; Kerr and Kelch 1999). According to the Nature Conservancy, Chinese privet is a major concern for forest ecosystems in Georgia, and the Florida Exotic Pest Council listed it as a Category 1 invasive species (The Nature Conservancy 2004; FLEPPC 1996).

The spread of Chinese privet has impending consequences, and it is evident that control needs to be implemented. One method may be by using lace bugs (Hemiptera: Tingidae) as a classical biological control agent. Although biocontrol is favored because it is considered to be environmentally safe and economically favorable, safety regulations have become increasingly stringent for pre release of organisms because of risks associated with introduced biocontrol agents on nontarget organisms and biodiversity (Simberloff and Stiling 1996). Tingids are useful for biological control because of their typical host specificity (Drake and Ruhoff 1965). However, lace bug host specificity sometimes extends to other members of a genus or plant family; for example, the azalea

lace bug, *Stephanitis pyrioides* Scott, primarily feeds on plants in the family Ericaceae, but there are limited reports of *S. pyrioides* extending feeding to different plant families (Nair and Braman 2012). To ensure relative host specificity and evaluate risks on nontarget organisms, host-specificity tests can be performed on candidates for biocontrol (Schroeder and Goeden 1986)

One candidate is *Leptoypha hospita* Drake et Poor (Hemiptera: Tingidae), found in China feeding on Chinese privet and causing plant dieback (Zhang et al. 2008).

L. hospita's potential raised the idea of studying native, congeneric lace bug species that currently utilize swamp privet (Oleaceae), *Foresteria acuminata* Michx, but do not use Chinese privet as a host (Mead 1975). The swamp privet is similar to the Chinese privet in size, growth habit and habitat but differs in that it is deciduous (Krüssmann 1986; Johnson and Hoagland 1999). Much like Chinese privet, it is found in swamps, wetlands, riparian forests and around ponds and lakes in the Southeastern United States (Johnson and Hoagland 1999). Lace bug species *Leptoypha ilicis* Drake, *Leptoypha elliptica* Mcatee (Wheeler 2002), and *Leptoypha mutica* Say (Mead 1975) all utilize the swamp privet as a host. *L. mutica* was the most commonly collected species in our area and became the concentration of our studies. The fringe tree, *Chionanthus virginicus* L., and green ash, *Fraxinus pennsylvanica* Marsh, both in the family Oleaceae, tribe Oleaeae, are also reported hosts of *L. mutica* (Drake 1918). The similarities between these Oleaceae plant species suggest potential for the native *Leptoypha* spp. to utilize the non-native Chinese privet. A better understanding of *Leptoypha* spp. and their plant host selection will aid in understanding how exotic plants interact with native herbivores and in selection of future candidates for biocontrol of invasive weeds.

Materials and Methods

Collection and rearing methods. A population of lace bugs was collected on 13 July and 19 August 2011 from the Montezuma Bluff Wildlife Management Area, Montezuma, GA (Macon Co., N32° 20.274', W084° 1.2245') off of native swamp privet and green ash using beat sheets and aspirators. Dense forest bounded by a river characterizes this sometimes-swampy area. A second population of lace bugs was collected in July 2011 from an established fringe tree in Spalding Co., GA (N33° 11.6638', W084° 8.7993').

Lace bugs were reared at the University of Georgia Griffin Campus, Griffin, GA (Spalding Co., N33° 15.6353', W084° 16.8139') with the two lace bug populations being kept separate. They were placed in plastic friction fitting petri dishes (17.78 cm diameter x 7.62 cm height) on small branches of swamp privet with the cut ends wrapped in moist paper towels. The lace bugs were maintained on a 14:10 (L:D) photoperiod at approximately 23°C. The swamp privet cuttings were difficult to maintain because the leaves would desiccate and fall off the stem within a few days, so fringe tree leaves (*Chionanthus virginicus*) became an alternative food source for rearing.

Samples from both lace bug populations were sent to the Smithsonian National Museum of Natural History for identification by Tom Henry. He identified all specimens as *Leptoypha mutica* in February 2012.

No choice test. A no choice study was conducted between 21 October and 15 November 2011 to determine acceptance and utilization of Chinese privet by the two lace bug populations. Lace bugs were added to 30 containers consisting of plastic friction fitting petri dishes 17.78 cm diameter x 7.62 cm height to which 4 lace bugs were added per container (2♀ and 2♂). Each container had either a fringe tree leaf, a 10 cm clipping

of new growth from swamp privet, or 10 cm clippings from Chinese privet. Each clipping or leaf had moist paper towels placed around their stems to maintain moisture. The study consisted of ten replications of each of the three plant species. Population 1 bugs, collected from fringe tree (Spalding Co.) were added to five of the ten replications, and population 2 lace bugs, collected from swamp privet (Macon Co.), were added to the remaining five replications. Experiments were conducted in a Percival growth chamber (Percival Scientific, INC.; Perry, IA; Model: I-36VL; S/N 3404.02.02B) at the University of Georgia Griffin campus set at a temperature of 27°C ($\pm 1^\circ$) and a 14:10 (L:D) photoperiod. Containers were then placed randomly in the growth chamber (Figure 2.1).

Water was added to the paper towel as needed every 2 to 3 days to keep the plants hydrated. Plant material that turned brown or desiccated was replaced with fresh cuttings or leaves. The old plant material was placed in a small petri dish, labeled accordingly. At the end of the 25 days, each dish was examined and the frass (feces) spots (upper leaf/lower leaf), eggs, and live bugs were recorded (Figure 2.1).

Laboratory choice tests. Lace bug preference and the extent of damage to Chinese privet and swamp privet were evaluated in two separate laboratory choice trials (combined in analysis) consisting of 24 replications each. Three additional trials (combined in analysis) comparing their choice among three plant species, Chinese privet, swamp privet and green ash were conducted and also replicated 24 times each. All trials were conducted in a Percival growth chamber at a temperature of $24 \pm 0.5^\circ\text{C}$ and a photoperiod of 14:10 (L:D) from 19 June to 2 August 2012. Plant species were arranged like spokes of a wheel in the same type of containers used in previous trials (figure 2.2). A single lace bug was placed in the middle of each petri dish (not on plants) containing

the plant species of interest. Twenty-four total lace bugs were used in each trial with 12 (6♀ and 6♂) being from population 1 lace bugs and 12 (6♀ and 6♂) being from population 2 lace bugs.

The containers were then randomly placed in the growth chamber. The stems of each cutting were wrapped in paper towels and kept moist daily. Data were collected daily and included the location of the lace bug and damage to plants on a scale of 1-10 (1 being least damaged). Trials 1 and 2 were three-day evaluations, and trials 3, 4 and 5 were seven-day evaluations.

Field choice test. This experiment took place at the University of Georgia Griffin Campus, Griffin, GA (Spalding Co.) in May 2013. Five (6 x 6 x 6', 20 x 20) Bioquip mesh Lumite portable field cages (Bioquip Products Inc., Rancho Dominguez, CA, 1406B) were set up in a shaded area under a pecan grove in a randomized block design (figure 2.3). Three different potted host plants were used as treatments: swamp privet, Chinese privet, and green ash. One of each host plant was randomly placed inside each cage. Fourteen male and 14 female *L. mutica* that had been reared on fringe tree leaves were added to each cage by putting them in small petri dishes placed on top of ≈ 12-inch tall boxes to discourage predation by any ground predators. Test plants were arranged so they were equidistant from the release point and each other. For seven days at ≈ 3:30 pm, plants were examined to determine the location of the lace bugs and for the extent of feeding damage estimated on each on a scale of 1 -10 (1=least damaged).

Statistical analysis. No choice test. Data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure, and means were separated with Fisher projected least significant difference (LSD) (SAS Institute 2010).

Laboratory choice tests. Data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure, and means were separated with Fisher projected least significant difference (LSD) test. Effects of gender and population were compared for plant choice and damage with trials 1 and 2 analyzed together and trials 3,4, and 5 analyzed together (SAS Institute 2010).

Field choice test. Data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure and means were separated with Fisher projected least significant difference (LSD) test (SAS Institute 2010).

Results

Collection and rearing methods. It appeared lace bug abundance was lower during the second collection from the Montezuma Bluff Wildlife Management Area (Macon Co.) and they were more common on lower vegetation in comparison to the previous month. Also, the lace bugs seemed to have moved to lower vegetation in comparison to the previous month where lace bugs were primarily on the higher limbs. This indicates a potential migration to the forest floor in preparation for the cooler months; therefore, the best time for collection would potentially be before the end of July in the southern Georgia area. The lace bugs were found mostly feeding on the upper surface of swamp privet and green ash (*Fraxinus pennsylvanica*).

No choice test. The two populations of lace bugs differed in their performance when the results for the three host plants were combined for analysis (Table 2.1). The location of frass is an indication of feeding sites. Frass on the upper ($F_{1,22} = 0.00$, $P = 0.957$) and lower ($F_{1,22} = 0.24$, $P = 0.628$) surface of the leaves and a combination of the frass ($F_{1,22} = 0.13$, $P = 0.71$) was similar for both populations. Bugs collected from fringe tree

(population 1) produced 4 times as many eggs as those collected from swamp privet (population 2) ($F_{1,22} = 6.32$, $P = 0.02$). Bugs collected from fringe tree (population 1) had a higher survival rate than bugs collected from swamp privet (population 2) living over 1.5 weeks longer, which could have contributed to the increased egg production ($F_{1,22} = 4.95$, $P > 0.037$).

Analysis of *L. mutica* performance on the three plant hosts showed that frass abundance was highest on the upper leaf surface of Chinese privet followed by swamp privet then fringe tree ($F_{2,22} = 17.31$, $P < 0.0001$; Table 2.2). In contrast, frass on the lower leaf surface did not differ among plant hosts ($F_{2,22} = 0.65$, $P = 0.530$). When upper and lower frass was combined there was no difference among plant hosts ($F_{2,22} = 1.33$, $P = 0.285$). *L. mutica* laid more eggs on swamp privet than on Chinese privet but not fringe tree ($F_{2,22} = 3.92$, $P = 0.035$). Lace bug survival was similar on all three host plants ($F_{2,22} = 2.38$, $P = 0.116$).

Significantly more frass was deposited on the upper leaf surface of Chinese privet than either fringe tree or swamp privet for bugs collected off of fringe tree (population 1) ($F_{2,8} = 17.59$, $P = 0.0012$; Table 2.3), but there were no differences in frass on the lower leaf surface or when upper and lower frass was combined ($F_{2,8} = 2.25$, $p = 0.168$; $F_{2,8} = 1.71$, $P = 0.241$). Lace bugs collected off of swamp privet (population 2) deposited significantly more frass on upper leaf surfaces of swamp privet and Chinese privet than fringe tree, while frass deposits on the lower leaf surfaces were the same among hosts ($F_{2,8} = 11.56$, $p = 0.004$; $F_{2,8} = 0.55$, $p = 0.598$). Upper/ lower frass combined was found equally among plant hosts ($F_{2,8} = 4.78$, $P = 0.050$).

Lace bugs from fringe tree (population 1) deposited more eggs on swamp privet than on either fringe tree or Chinese privet despite equal survival on all hosts ($F_{2,8} = 9.37$, $p = 0.008$; $F_{2,8} = 1.56$, $p = 0.2687$; Table 2.3). Egg production and survival were not significantly different among any of the hosts for bugs collected from swamp privet (population 2) ($F_{2,8} = 0.67$, $p = 0.539$; $F_{2,8} = 1.07$, $p = 0.387$).

When fringe tree was the host plant, no differences in the amount of frass on the upper leaf surface, lower leaf surface, upper/ lower frass combined or survival were observed between populations ($F_{1,4} = 2.80$, $p = 0.169$; $F_{1,4} = 2.69$, $p = 0.176$; $F_{1,4} = 2.80$, $p = 0.169$; $F_{1,4} = 2.67$, $p = 0.178$; Table 2.4). Likewise, both populations performed similarly on swamp privet, with no differences in the amount of frass on the upper leaf surface, lower leaf surface, upper and lower frass combined or survival ($F_{1,4} = 5.21$, $p = 0.085$; $F_{1,4} = 1.90$, $p = 0.241$; $F_{1,4} = 7.12$, $P = 0.056$; $F_{1,4} = 1.45$, $p = 0.294$). Egg production, however, was significantly greater in population 1 lace bugs collected from fringe tree than population 2 lace bugs, which were originally collected from swamp privet ($F_{1,4} = 12.82$, $p = 0.023$). Lace bugs from both populations had similar levels of frass on upper leaf surfaces ($F_{1,4} = 0.68$, $p = 0.457$), lower leaf surfaces ($F_{1,4} = 0.22$, $p = 0.665$), and upper/ lower frass combined ($F_{1,4} = 0.00$, $P = 0.973$) on Chinese privet and they laid equal numbers of eggs ($F_{1,4} = 0.00$, $p = 1.00$) and survived equally well on it ($F_{1,4} = 2.67$, $p = 0.178$).

Laboratory choice tests.

Trials 1 & 2. Lace bugs were observed most frequently on the petri dish container (n=144 observations), and were found in equal numbers on Chinese and swamp privet ($F_{2,137} = 3.90$, $P = 0.023$; Table 2.5), which was reflected in feeding damage scores that

did not differ between the two hosts Chinese privet and swamp privet ($F_{1,90} = 0.04$, $P = 0.842$). Lace bug population source ($F_{2,137} = 0.12$, $P = 0.735$) and gender ($F_{2,137} = 0.03$, $P = 0.865$) had no effect on location on plant or container, or damage (population $F_{1,90} = 0.20$, $P = 0.660$; gender $F_{1,90} = 2.74$, $P = 0.102$).

Trials 3-5. Lace bugs were found most often on green ash and were found similarly among swamp privet, Chinese privet or the cage ($F_{3,279} = 36.28$, $P < 0.0001$; Table 2.5). Feeding damage was highest on green ash and swamp privet followed by Chinese privet ($F_{2,208} = 6.81$, $P = 0.001$). Neither population nor gender affected lace bug location (population $F_{1,279} = 1.88$, $P = 0.172$; gender $F_{1,279} = 2.04$, $P = 0.154$) or damage (population $F_{1,208} = 0.07$, $P = 0.785$; gender $F_{1,208} = 0.28$, $P = 0.595$).

Field studies for host plant suitability – choice test. The field choice test, confirmed laboratory results with lace bugs being observed most frequently and causing the most damage on green ash, and no significant difference between swamp privet and Chinese privet (location $F_{2,8} = 20.31$, $P = 0.0007$; damage $F_{2,8} = 76.63$, $P = 0.0001$; Table 2.6).

Discussion

Original collections and observations of the two lace bug populations suggested they could have been two different lace bug species. The lace bugs collected from swamp privet (Macon Co.) appeared slightly smaller in size and more mobile than those collected off of fringe trees (Spalding Co.). Also, Wheeler (2002) suggested lace bugs collected from swamp privet could be either *L. elliptica* McAtee or *L. ilicis* Drake, mesophyll feeders known to inhabit swamp privet. However, the two lace bug populations used in this study were identified in February 2012 as *L. mutica* (personal communication Tom Henry USDA/ARS/SEL) and they behaved similarly throughout the

studies. The no-choice test general comparison of the two lace bug populations (table 2.1) showed no differences in their performance except for more egg production by population 1. This, however, was probably a direct result of their higher survival in that test. The laboratory host choice test also supports their identification as one species in that there were no differences between the populations and their host choice.

Prior to this study, Chinese privet was not a confirmed host of *L. mutica*, although it was known to utilize other members of the Oleaceae family including fringe tree, green ash, and swamp privet (Drake 1918; Mead 1975). The no choice test showed that *L. mutica* adults could survive, feed, and lay eggs on Chinese privet. Results did indicate less egg production on Chinese privet (tables 2.2 and 2.3). This could mean that lace bugs do not obtain the same amount of energy from Chinese privet, so it was less acceptable or simply the lace bugs used were unable to produce eggs. Also, observations of egg placement on the different plant hosts showed occasional exposed eggs on the Chinese privet, whereas egg placement on other hosts was deeper in the plant tissue (unpublished data). These observations could be clues to as why Chinese privet is not a preferred host plant. The nutritional composition of Chinese privet leaf tissue may not be optimum for *L. mutica* fitness, or Chinese privet leaves could be an unsuitable host for best egg placement and development. The optimal oviposition theory states that females will lay their eggs where their offspring will be able to perform best (Jaenike 1978); also, plant surfaces are one of the most critical confluences affecting host acceptance by insects (Eigenbrode and Espelie 1995). For example, the azalea lace bug, *Stephanitis pyrioides*, is thought to consider hosts based on leaf wax chemistry (Wang et al. 1999), leaf pubescence (Schultz 1993), stomatal characters (Kirker et al. 2008), and leaf moisture

content (Wang et al.1998), so even among azalea cultivars there are different levels of resistance. These selection criteria can be accredited to why tingids tend to be host specific and why *L. mutica* does not host shift in the wild to the ever-abundant Chinese privet.

In a small trial 5 male and 5 female lace bugs were caged with Chinese privet from 14 December 2011 to 29 March 2012 at which time there were over 100 adults (unpublished data). Therefore, the lace bugs have the potential to utilize Chinese privet, but we have never observed significant infestation in the wild on Chinese privet. Our studies suggest that the reason *L. mutica* does not readily utilize Chinese privet is because it is not a preferred host. When given only the choice of swamp privet and Chinese privet, the lace bug did not prefer one to the other, but whenever green ash was present the lace bugs always preferred it. In most forest areas where *L. mutica* were established green ash was also present, so it is likely that ash was the primary host supporting the population. This restricted host preference makes *L. mutica* an unlikely candidate for control of Chinese privet without a host shift, but it is encouraging for the similar Asian lace bug, *L. hospita*'s potential as a biological control agent. Chinese privet is the preferred host of this lace bug, and if released, acting similarly, it will not shift to other native plant hosts.

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Table 2.1. Mean (\pm S.E.) number of *L. mutica* frass spots on upper, lower and combined leaf surfaces, and eggs laid, and survival of lace bug from two populations during no choice laboratory assays in which they were limited to one of three hosts. Numbers are the means for all three hosts combined.

Population	Frass (upper)	Frass (lower)	Frass (combo)	Eggs	Survival
1*	100.13 \pm 16.9 A	122.27 \pm 26.0 A	222.40 \pm 27.2 A	8.87 \pm 2.8 A	3.53 \pm 0.2 A
2*	101.00 \pm 15.9 A	107.07 \pm 14.9 A	208.07 \pm 26.5 A	2.20 \pm 0.8 B	2.87 \pm 0.3 B

Means in the in the same column bearing the same letters are not significantly different ($\alpha= 0.05$);

* Population 1= Fringe tree lace bug, Population 2= Swamp privet lace bug

Table 2.2. Mean (\pm S.E.) number of *L. mutica* frass spots on upper, lower, and combined leaf surfaces, and eggs laid, and survival of lace bugs on three hosts during no choice laboratory assays in which they were limited to one of three hosts.

Plant Host	Frass (upper)	Frass (lower)	Frass (combo)	Eggs	Survival
Fringe tree	44.90 \pm 7.90 C	139.20 \pm 33.8 A	184.10 \pm 34.0 A	4.80 \pm 2.1 AB	2.80 \pm 0.3 A
Swamp privet	95.70 \pm 15.6 B	106.70 \pm 17.8 A	202.40 \pm 30.3 A	10.40 \pm 3.8 A	3.20 \pm 0.3 A
Chinese privet	161.1 \pm 14.4 A	98.10 \pm 23.5 A	259.20 \pm 31.0 A	1.40 \pm 0.5 B	3.60 \pm 0.2 A

Means in the in the same column bearing same letter are not significantly different ($\alpha= 0.05$)

Table 2.3. Mean (\pm S.E.) number of *L. mutica* frass spots on upper, lower, and combined leaf surfaces, and eggs laid, and survival of lace bugs from two populations during no choice laboratory assays in which they were limited to one of three hosts.

Plant Host	Frass (upper)	Frass (lower)	Frass (Combo)	Eggs	Survival
Population 1*					
Fringe tree	56.40 \pm 12.2 B	192.20 \pm 60.2 A	248.60 \pm 23.9 A	6.00 \pm 3.7 B	3.20 \pm 0.4 A
Swamp privet	68.60 \pm 7.20 B	89.20 \pm 27.5 A	157.80 \pm 13.2 A	19.20 \pm 5.0 A	3.60 \pm 0.2 A
Chinese privet	175.40 \pm 26.0 A	85.40 \pm 29.7 A	260.80 \pm 22.2 A	1.40 \pm 0.6 B	3.80 \pm 0.2 A
Population 2*					
Fringe tree	33.40 \pm 8.00 B	86.20 \pm 10.2 A	119.60 \pm 7.41 A	3.60 \pm 2.2 A	2.40 \pm 0.6 A
Swamp privet	122.80 \pm 26.1 A	124.20 \pm 22.6 A	247.00 \pm 21.3 A	1.60 \pm 0.5 A	2.80 \pm 0.5 A
Chinese privet	146.80 \pm 12.6 A	110.80 \pm 39.1 A	257.60 \pm 19.3 A	1.40 \pm 0.9 A	3.40 \pm 0.2 A

Means within populations and in the in the same column bearing the same letter are not significantly different ($\alpha= 0.05$);

* Population 1= Fringe tree lace bug, Population 2= Swamp privet lace bug

Table 2.4. Mean (\pm S.E.) number of *L. mutica* frass spots on upper, lower, and combined leaf surfaces, and eggs laid, and survival of lace bugs from two populations during no choice laboratory assays in which they were limited to one of three hosts.

Population	Frass(upper)	Frass (lower)	Frass (combo)	Eggs	Survival
Fringe tree					
1*	56.40 \pm 12.2 A	192.20 \pm 60.2 A	248.60 \pm 23.9 A	6.00 \pm 3.7 A	3.20 \pm 0.4 A
2*	33.40 \pm 8.0 A	86.20 \pm 10.2 A	119.60 \pm 7.42 A	3.60 \pm 2.2 A	2.40 \pm 0.6 A
Swamp privet					
1*	68.60 \pm 7.2 A	89.20 \pm 27.5 A	157.80 \pm 13.2 A	19.2 \pm 5.0 A	3.60 \pm 0.2 A
2*	122.80 \pm 26.1 A	124.20 \pm 22.6 A	247.00 \pm 21.3 A	1.60 \pm 0.5 B	2.80 \pm 0.5 A
Chinese privet					
1*	175.40 \pm 26.0 A	85.40 \pm 29.7 A	260.80 \pm 22.2 A	1.40 \pm 0.6 A	3.80 \pm 0.2 A
2*	146.80 \pm 12.6 A	110.80 \pm 39.1 A	257.60 \pm 19.3 A	1.40 \pm 0.9 A	3.40 \pm 0.2 A

Means for populations in the same column and the same host bearing the same letter are not significantly different ($\alpha= 0.05$);

* Population 1= Fringe tree lace bug, Population 2= Swamp privet lace bug

Table 2.5. Laboratory choice tests of *L. mutica* adults given the choice of two or three hosts. Means \pm S.E for adult location and percent leaf area damaged

Treatments	Trials 1 & 2		Trials 3-5	
	Location of lace bug	Leaf area damaged	Location of lace bug	Leaf area damaged
Swamp privet	0.85 \pm 0.1 B	13.42 \pm 0.1 A	2.13 \pm 0.2 B	22.94 \pm 0.2 A
Chinese privet	0.77 \pm 0.1 B	13.33 \pm 0.2 A	1.22 \pm 0.2 B	15.58 \pm 0.2 B
Container	1.29 \pm 0.1 A	-----	1.96 \pm 0.2 B	-----
Green ash	-----	-----	7.51 \pm 0.9 A	25.07 \pm 0.3 A

Means in the in the same column bearing different letters are significantly different ($\alpha= 0.05$)

Table 2.6. Field choice test assays with lace bug means \pm S.E for adult location and percent leaf area damaged

Treatments	Location of lace bug	Leaf area damage
Swamp privet	12.40 \pm 0.8 B	39.69 \pm 3.5 B
Chinese privet	5.40 \pm 0.4 B	31.76 \pm 1.1 B
Green ash	51.00 \pm 0.5 A	65.66 \pm 3.1 A

Means in the in the same column bearing different letters are significantly different ($\alpha= 0.05$)



Figure 2.1. No choice test of two *L. mutica* populations collected from fringe tree or swamp privet in an experimental growth chamber using three plant hosts (fringe tree, swamp privet, and Chinese privet).



Figure 2.2. Laboratory study testing *L. mutica* host choice among three plant hosts (swamp privet, Chinese privet, and green ash).



Figure 2.3. Field host choice test of *L. mutica* testing their response to intact potted plants of three different plant hosts (swamp privet, Chinese privet, and green ash).

CHAPTER 3

EVALUATION OF THE DEVELOPMENTAL
BIOLOGY OF *LEPTOYPHA MUTICA*

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ABSTRACT The native lace bug, *Leptoypha mutica* Say (Hemiptera: Tingidae), has demonstrated potential as an insect biological control agent of invasive Chinese privet (*Ligustrum sinense* Lour). To better evaluate the lace bugs' potential to establish on a new host, their developmental biology on Chinese privet was studied at different temperatures: 20°C, 24°C, 28°C, and 32°C. The complete duration of development of *L. mutica* from egg deposition through five instars on this previously unreported host ranged from 24.4 to 57.1 days. Estimated threshold temperatures (T_o) and calculated thermal unit requirements (K) for development of egg, nymphal and complete development were 11.0°C, 9.9°C, 10.5°C and 211.9, 326.8, 527.4 degree-days (DD). Results of this study indicated that *L. mutica* will oviposit and can develop successfully on Chinese privet over a wide range of temperatures and thermal unit requirements for development suggest the potential for multiple generations to occur in a single season.

KEY WORDS *Leptoypha mutica*, fringe tree lace bug *Ligustrum sinense*, Chinese privet, lace bug biology, developmental biology, invasive, biological control.

Chinese privet (Oleaceae), *Ligustrum sinense* Lour, was introduced into the United States in 1852 as an ornamental shrub (Dirr 1983) and escaped cultivation by 1932 (Small 1933). Because of its adaptations to a wide range of environments and habitats, Chinese privet has become a widespread, exotic plant found in almost all the southeastern United States. Even though the plant species has tropical origins, it can endure a range of light, temperature and soil conditions (Brown and Pezeshki 2000). Surveys show that of the ≈ 7.1 million hectares where the privet occurs it densely covers ≈ 1 million hectares (Miller et al. 2008; Rudis et al. 2006). In these areas, the privet depletes native plant diversity by competing for water and nutrients in the soil and by competing for light by producing a shade cover over forest understory (Morris et al. 2002; USDI Fish and Wildlife Service 1992; Merriam & Feil 2002). Other risks are decreased invertebrate communities and toxic affects on humans and animals (Biesmeijer et al. 2006; Crisp et al. 1998; Kerr and Kelch 1999). Chinese privet is listed as a category 1 invasive species according to The Florida Exotic Pest Council, and according to the Nature Conservancy is a major concern for forest ecosystems in Georgia (FLEPPC 1996; The Nature Conservancy 2004).

Chinese privet is an unrelenting problem with negative consequences and needs to be controlled. Once the privet is established it becomes difficult to remove and large-scale removal becomes labor intensive and requires the use of herbicides (Hanula et al. 2009). To help alleviate the spread, a potential solution is using lace bugs (Hemiptera: Tingidae) as a biological control agent. A U.S – China cooperative project on biological control of Chinese privet was initiated in 2005. In China, insects feeding on Chinese privet were surveyed and *Leptoypha hospita* Drake et Poor (Hemiptera: Tingidae) was

found as a potential biocontrol agent because of its common feeding on the privet that sometimes resulted in plant dieback (Zhang et al. 2008; Zhang et al. 2011). *L. hospita*'s promising potential as a control agent introduced the idea of further examination of native, congeneric lace bug species, *Leptoypha mutica* Say, currently utilizing swamp privet (Oleaceae), *Foresteria acuminata* Michx (Mead 1975). Other hosts of *L. mutica* include fringe trees, *Chionanthus virginicus* L., and green ash, *Fraxinus pennsylvanica* Marsh, both in the family Oleaceae (Drake 1918).

In some cases of classical weed control, just one or a combination of host specific biological control agents are released as controls (McFadyen 2003), but some times only a small proportion of multiple releases prove to be effective (Myers 1985; McEvoy and Coombs 2000; Denoth et al. 2002). In efforts to prevent futile implements of control, the candidate of interest should be extensively evaluated. The objective of this study was to better understand the relationship between temperature and development of *L. mutica* on Chinese privet. Knowing this relationship and developing a degree – day (DD) model can be used as prediction of occurrence and sustainability of the lace bug. The interrelationship between insects and invasive plants may also lead to clues of why *L. mutica* hasn't already taken advantage of the naïve host, Chinese privet. By better understanding the *Leptoypha* spp. and its biology, itself and future candidates for biocontrol of Chinese privet may be better understood and applied.

Materials and Methods

L. mutica adults used for this study were collected from fringe trees (*Chionanthus virginicus*) in Spalding Co. (N33° 11.6638', W084° 8.7993') and green ash (*Fraxinus pennsylvanica*) and swamp privet (*Foresteria acuminata*) from the Montezuma Bluff

Wildlife Management Area (Macon, Co., N32° 20.274', W084°). The lace bug collections were reared in the laboratory on fringe leaves in plastic petri dishes at a 14:10 (L:D) photoperiod at $\approx 23^{\circ}\text{C}$ on the University of Georgia, Griffin campus (Spalding Co., N33° 15.6353', W084° 16.8139').

Cuttings of Chinese privet (*Ligustrum sinense*) were obtained from potted greenhouse plants grown from field-collected seedlings. Cuttings were placed in cages constructed from 1 oz. diet cups (Jetware PC-100). Cages consisted of a water - filled diet cup with a lid with a small hole. A piece of Tygon tubing was placed in the hole, and the plant cutting was inserted into the tube and secured with Parafilm wrap. Several male and female lace bugs (≈ 10 total) were added to each plant cutting, and another diet cup with a screen bottom was put on top and secured with Parafilm (Figure 3.1). Cages were placed in Percival environmental growth chambers (Percival Scientific, INC.; Perry, IA; Model: I-36VL; S/N 3404.02.02B) at 4 separate temperatures ($\pm 0.5^{\circ}\text{C}$): 20, 24, 28, and 32°C . Females were allowed to oviposit for 24 hours at each experimental temperature then all lace bugs on the cuttings were moved to new cages with fresh cuttings of Chinese privet, and the process repeated.

To determine the development time of each life stage, all cages were monitored twice a day (10 am and 4 pm EST) under a dissecting microscope for the emergence of nymphs. As first instars emerged, they were transferred individually onto separate caged cuttings of Chinese privet in bug condos and monitored for development. Nymphal molts were evidenced by the presence of exuviae. The nymphs were reared and examined until adults emerged, and adult sex ratio was determined and any gender influence on duration of development was examined.

Statistical analysis. The mean duration was calculated for each life stage for total nymphal and for complete development. The effect of sex of the lace bug on development was compared at each temperature using the t – test procedure (PROC T-test; SAS institute 2010).

Least squares linear regression was used to establish the relationship between development rate (days) and temperature (°C) over the linear portion of development curves. The lower temperature threshold (T_o) was found by using the regression procedure, PROC REG (SAS institute 2010), on the developmental means to calculate the slope and intercept of the regression equation $Y = a + bX$. The equation was solved using the slope and intercept at no development ($Y = 0$), which produced the lower temperature threshold (T_o). The mean thermal unit requirement (K_t) for each life stage was calculated using the means at each temperature of all stages of development with the following equation:

$$K_t = (T - T_o) * D_t$$

Where T was either 20 °C, 24 °C, 28 °C, or 32 °C, T_o was the lower temperature threshold of a particular stage of interest, D_t was the mean development time (days) for a particular stage at temperature T . Results for each temperature at each life stage were then averaged to produce the final mean thermal unit requirements.

Results

Eggs of *L. mutica* were tubular, slightly curved, and yellow (Image 3.2). The cephalic pole was characterized by an oval shaped operculum, which was still visible once the eggs were inserted into the plant material. Eggs were found inserted on both the dorsal and ventral sides of leaves and commonly along the midrib. Eggs were

occasionally found in the petiole and stems as well. This occurred mostly in new growth where the plant tissue was still soft. Eggs developed successfully at all four temperatures. Eggs developed between 10.0 to 24.6 days with decreasing temperatures (Table 3.1). A little less than half of the total development was spent in the egg stage.

There are five nymphal stages of *L. mutica*. The nymphs were oblong and flat. Each stage could be identified by the presence of exuviae on the plant material after molting. Newly emerged nymphal instars were reddish in color with red eyes, but turned pale yellow/brown in color with black eyes after \approx 1 day (Figure 3.2). The nymphs had short setae on their dorsal surface. They fed on the ventral and dorsal epidermis of the Chinese privet leaves almost immediately after eclosion. Their feeding on the mesophyll tissue produced chlorotic bleached appearing leaves. Total nymphal development ranged from 14.7 to 33.9 days as temperatures decreased (Table 3.1).

The adult lace bugs were elongate and light brown/ cream with red eyes upon emergence. Their color changed to a deeper brown color and black eyes with a short time (\approx 1 day). Males could be distinguished by their pinched, narrow abdomens, whereas females had a fuller, rounded abdomen with an ovipositor present (Figure 3.3). The duration of complete development to adulthood ranged from 24.4 to 57.1 days with decreasing temperature (Table 3.1). The lace bugs were able to develop at all temperatures, but had the least success at 20°C with only 2 observations, 7.7% survival (N = 26). The optimal temperature for complete development was 28°C with 53 observations, 52.0% survival (N=102) to the adult stage. Female and male development did not differ significantly at any temperature for any life stage ($\alpha > .05$). The male/

female sex ratio for 20°C was 1:1, 24°C was 31:24, 28 °C was 27:28, and 32 °C was 15:14.

The lower threshold temperatures (T_0) for egg development was 11.0°C, 6.2°C for first instar development, 14.9°C for second instar development, 13.8°C for third instar development, 13.0°C for fourth instar development, 9.8°C for fifth instar development, 9.9°C for total nymphal development, and 10.5°C complete development. Mean thermal unit requirement (K_i) for egg development was 211.9 degree-days (DD), 90.0 DD for first instars, 39.8 DD for second instars, 40.8 DD for third instars, 49.6 DD for fourth instars, 86.9 for fifth instars, 326.8 DD for total nymphal, and 527.4 DD for complete development. If lines are backward extrapolated on figure 3.4 they predict the stated the values (Table 3.2; Figure 3.4).

Discussion

Studying the basic biology of *L. mutica* is useful not only to better understand the behavior of the lace bug, but also to analyze its full potential as a biological control agent. The lace bugs were able to develop at all temperatures, but were least successful at surviving at 20°C. Other factors could have contributed to mortality other than temperature alone such as damage while transferring first instars. The regression equations for the reciprocal of developmental times on temperature for each life stage and values for T_0 and K were used to predict at what temperature each life stage would cease to develop, and it was estimated that complete development would cease below 10.5°C.

This relatively low threshold and having a broad range over which development occurs, suggests the potential utility of this insect under a variety of environmental conditions. Currently in the United States, Chinese privet is found from Texas to Florida,

all the way north to the New England states (USDA – NRCS 2002). *L. mutica* is found in a similar range having been reported from Maine, west to North Dakota, and south to Florida and Texas (Mead 1975).

Developmental work done at 24 – 26°C on Asian lace bug, *L. hospita*, indicates total development occurred in 24.77 days on Chinese privet (Zhang et al. 2011) in comparison to the *L. mutica* at 34.2 days when development times at 24°C and 28°C are averaged. Barber and Weiss (1922) indicated that *L. mutica* probably has two generations per year and overwinter as adults. If a host shift did occur, their relatively short generation time and multiple broods would aid in their ability to potentially heavily colonize Chinese privet and control it.

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Table 3.1. Mean \pm S.E duration of development in days, and number of individuals completing each stage, of *Leptoypha mutica* on *Ligustrum sinense* cuttings.*

Temp.°C	Egg	1	2	Instar			5	Nymphal	Total Complete
				3	4				
20 °C	24.6 \pm 0.4 (26)	4.3 \pm 1.1 (6)	9.3 \pm 2.3 (5)	6.5 \pm 0.3 (7)	6.4 \pm 0.8 (8)	9.6 \pm 0.9 (6)	33.9 \pm 2.8 (2)	57.1 \pm 2.8 (2)	
24 °C	15.8 \pm 0.1 (171)	5.2 \pm 0.2 (68)	3.7 \pm 0.2 (69)	3.7 \pm 0.1 (71)	4.6 \pm 0.2 (67)	5.9 \pm 0.3 (61)	22.8 \pm 0.5 (54)	38.6 \pm 0.5 (53)	
28 °C	12.4 \pm 0.2 (94)	4.9 \pm 0.2 (68)	2.7 \pm 0.2 (64)	2.8 \pm 0.1 (60)	3.2 \pm 0.2 (57)	4.6 \pm 0.2 (56)	17.6 \pm 0.3 (53)	29.8 \pm 0.4 (53)	
32 °C	10.0 \pm 0.2 (58)	3.8 \pm 0.2 (47)	2.5 \pm 0.2 (43)	2.5 \pm 0.2 (40)	2.9 \pm 0.2 (36)	3.7 \pm 0.3 (29)	14.7 \pm 0.5 (29)	24.4 \pm 0.6 (28)	

*Values in parentheses are numbers of individuals entering each stage

Table 3.2. Linear thermal unit models, threshold temperatures (T_0), and mean thermal unit requirement (K) for development in degree days (DD) of each stage of *Leptopypha mutica* (See text for discussion of calculation of T_0 and K).

Developmental Stage	Equation and r^2 *	T_0 ($^{\circ}\text{C}$)	K(DD)
Egg	$y = -0.054t + 0.0049$ $r^2 = 0.72$	11.0	211.9
Instars			
First	$y = -0.074t + 0.012$ $r^2 = 0.12$	6.2	90.0
Second	$y = -0.541t + 0.036$ $r^2 = 0.16$	14.9	39.8
Third	$y = -0.408t + 0.030$ $r^2 = 0.20$	13.8	40.8
Fourth	$y = -0.320t + 0.025$ $r^2 = 0.12$	13.0	49.6
Fifth	$y = -0.138t + 0.014$ $r^2 = 0.12$	9.8	86.9
Total nymphal	$y = -0.032t + 0.003$ $r^2 = 0.58$	9.9	326.8
Complete	$y = -0.020t + 0.0019$ $r^2 = 0.75$	10.5	527.4

* y, reciprocal of mean developmental times; t, temperature; r^2 , coefficient of correlation



Figure 3.1. Cages used for developmental studies. Plant material was inserted through tube into lid covered water - filled diet cup. Parafilm was wrapped around the stem and tube, so insects could not get into the water cup. The bottom of another diet cup was cut out and covered with screen and then placed upside down on top of the water cup with cutting. The two cups were secured together with Parafilm.

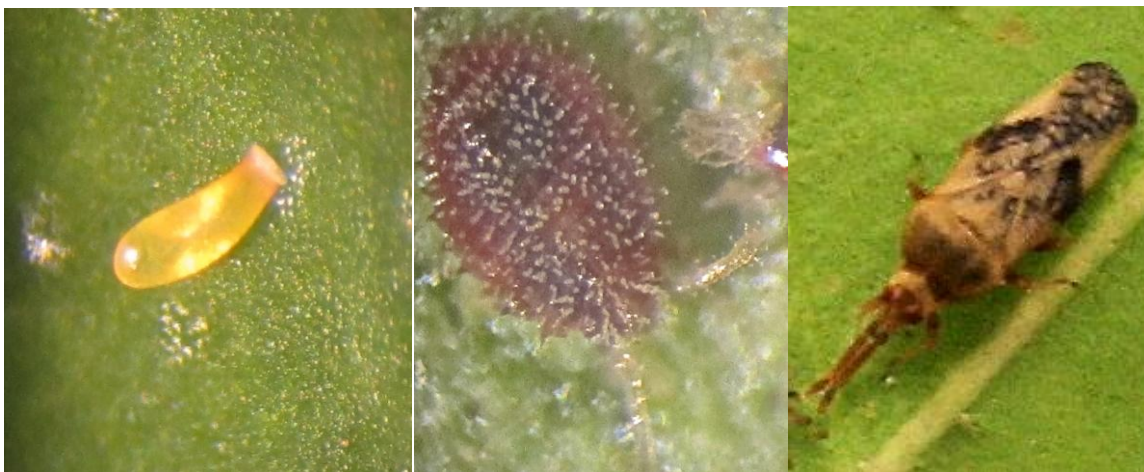


Figure 3.2. Life stages of *L. mutica*: egg (left), nymph (center), adult (right).



Figure 3.3. Gender of *L. mutica* determined by females having rounded abdomen with ovipositor (pictured left) and males having pinched abdomens (pictured right)

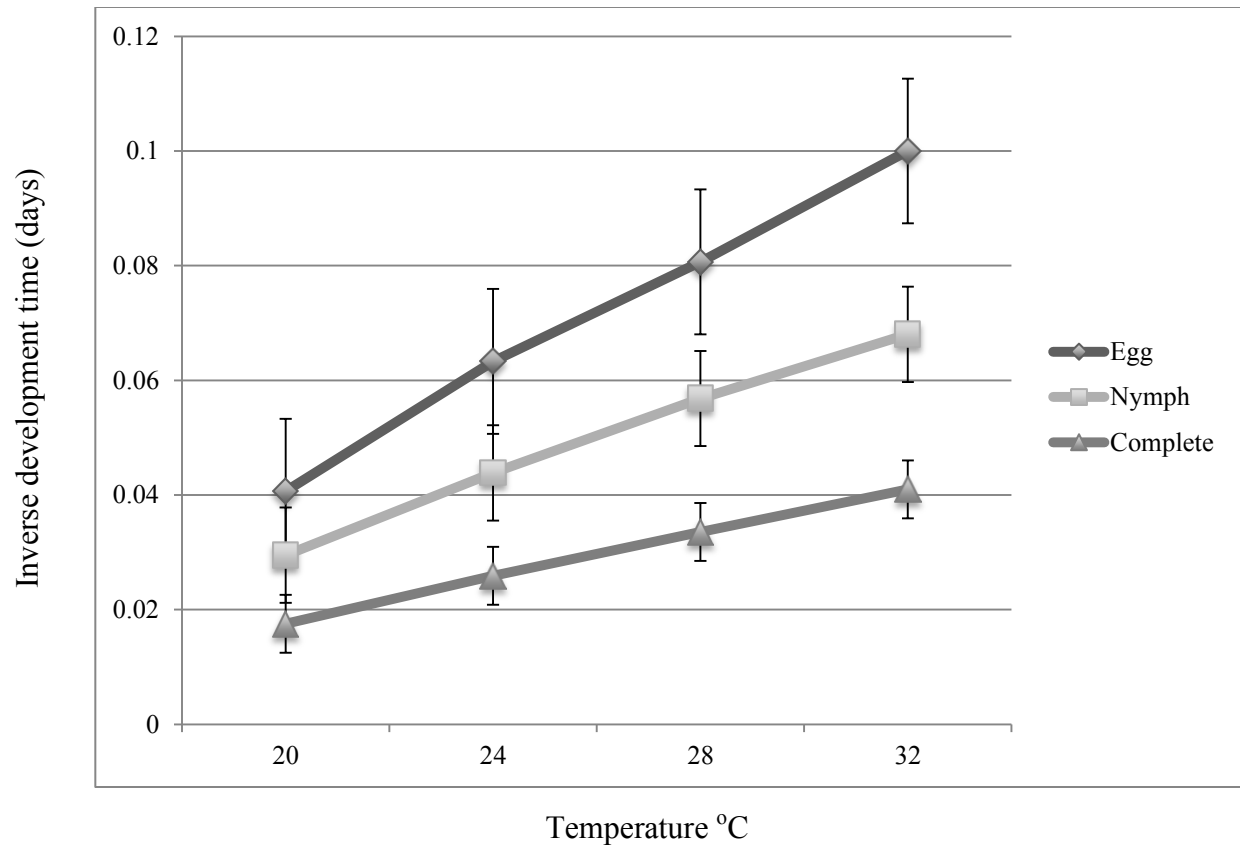


Figure 3.4. Prediction of developmental thresholds based on inverse developmental means for time (in days) for egg to hatch, total nymphal development, and complete development.

CHAPTER 4

NATURAL ENEMIES OF *LEPTOYPHA MUTICA*

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ABSTRACT In the southeastern United States, Chinese privet (*Ligustrum sinense* Lour) is a critically invasive plant species causing depletion of native diversity. One method of control may be through using the native lace bug *Leptoypha mutica* Say as a biological control agent. The native lace bug does not commonly feed on Chinese privet in the wild, and it is thought that this may be due to natural enemies associated with Chinese privet. Beat sampling to assess predator populations was done on different plant species swamp privet (*Foresteria acuminata* Michx), green ash (*Fraxinus pennsylvanica* Marsh), both of which support the lace bug, and Chinese privet at different times and locations. A survey of arthropods inhabiting Chinese privet was also done by administering beat sampling to 358 Chinese privet plants in varying locations. Surveys for egg parasitoids were done by deploying sentinel plants (Chinese privet and green ash) with *L. mutica* eggs into site of known lace bug activity. The plants were recovered and observed. Results from the beat samples did not show a higher natural enemy complex associated with Chinese privet than other plant hosts. One mymarid wasp was recovered from the egg parasitoid samples.

INDEX WORDS: Chinese privet, *Ligustrum sinense*, *Leptoypha mutica*, biological control, invasive, natural enemy complex, egg parasitoids, sentinel plants.

Invasive species cost the United States billions of dollars every year through prevention, detection, response, control, research, outreach, and habitat restoration. (USDI Fish & Wildlife Service 1992). Chinese privet (Oleaceae), *Ligustrum sinense* Lour, is an invasive plant species in the southeastern United States found on ≈ 7.1 million hectares where it densely covers ≈ 1 million hectares (Miller et al. 2008; Rudis et al. 2006). Its aggressive spread can be attributed to its ability to tolerate a range of light, temperature and soil conditions (Brown and Pezeshki 2000). It is an evergreen that can grow up to 10 m in height with prolific seed production and vegetative reproduction (Miller 2003). The exotic privet outcompetes native plants for water, nutrients and light (Morris et al. 2002; Biesmeijer et al. 2006), decreases invertebrate communities and causes potential toxic effects to humans and animals (Crisp et al. 1998; Kerr and Kelch 1999).

Because large-scale control of Chinese privet is labor intensive and requires the use of herbicides, biological control has been turned to in order to alleviate some of the pressures employed by Chinese privet's invasion (Hanula et al. 2009). Through a U.S. – China cooperative study, surveys in China of insects feeding on Chinese privet suggested *Leptoypha hospita* Drake et Poor (Hemiptera: Tingidae) as a potential biological control agent (Zhang et al. 2008). These studies raised the question of why native, congeneric lace bug species, *Leptoypha mutica* Say, currently utilizing swamp privet (Oleaceae), *Foresteria acuminata* Michx, do not move to Chinese privet (Mead 1975). *L. mutica* is also hosted by fringe trees, *Chionanthus virginicus* L., and green ash, *Fraxinus pennsylvanica* Marsh, both in the Oleaceae family (Drake 1918). It is thought that maybe natural enemy complexes associated with Chinese privet are preventing the colonization

of the host plant. Tingids are not known to have many obligate predators or parasitoids (Oliver et al. 1985), but some activity has been reported on eggs of the andromeda lace bug, *Stephanitis takeyai* Drake and Maa, by the mymarid egg parasitoid *Anagrus takeyanus* Gordh (Hymenoptera) in Connecticut (Dunbar 1974, Gordh & Dunbar 1977). Parasitism of azalea lace bug, *Stephanitis pyrioides* Scott, eggs by *A. takeyanus*, was reported by Braman et al. (1992), while Balsdon et al. (1996) elucidated parasitoid biology and seasonal dynamics in Georgia. Sometimes, despite host preference, females will select hosts where progeny are less vulnerable to natural enemies (Jeffries and Lawton 1984). By better understanding the *Leptoypha* spp. and its host plants' natural enemy complexes, better knowledge of why native lace bugs do not utilize Chinese privet in the wild can be gained and potential predators and parasites that could limit *L. hospita* establishment on Chinese privet can be identified before its potential release.

Materials and Methods

Field study observations of natural enemies on different plant species. A part of this study was done in the Montezuma Bluff Wildlife Management area in Montezuma, Ga (Macon, Co., N32° 20.274', W084° 1.2245') on 21 June 2012. This area can be described as riparian forest alongside the Flint river and is known to contain the *Leptoypha* spp. Sampling was done on three different known host species of *L. mutica*; green ash (*Fraxinus pennsylvanica*), swamp privet (*Forestiera acuminata*), and Chinese privet (*Ligustrum sinense*). Natural enemy comparison among the three plant species was evaluated by administering beat sampling (5 beats) to 40 plants of each species. The identification of the specimen and number of each was recorded per plant type by on site visual observation.

Additional sampling was done in a riparian forest alongside the Oconee River 12 June 2013 at the State Botanical Garden in Athens, GA (Clarke Co., N33°54.046', W083°22.435'), which is also inhabited by *L. mutica*. Forty beat sampling (5 beats/ sample) were collected per species from green ash and Chinese privet. This beat sampling was done over pans filled with seventy percent alcohol in hopes to catch more specimens and be able to better identify them. The samples were then poured into specimen cups and later examined for identification of specimen and numbers of each. This sampling procedure was repeated at the Montezuma Bluff Wildlife Management area on 21 June 2013.

Field survey of arthropods on *Ligustrum sinense*. Sampling took place at several locations in Spalding County, GA on two different dates: 9 July 2012 and 18 July 2012. On each date, beat samples (5 beats) were collected from 10 Chinese privet plants ranging from ~ 30.48 cm to 1.5 m. This was repeated 4 times in different areas encompassing a variety of different habitats. On 9 July, samples were taken from four habitats (N33°11.6638', W084°8.7993'): fence line, creekside, forest interior, and forest edge. On 18, samples were taken from four areas: a swamp (N33°19.1678', W084°19.6643), a fence line (N33°19.1678', W084°19.6643), a lakeside (N33°17.0107', W084°20.9091'), and a field edge (N33°15.6353', W084°16.8139'). The number and type of arthropod specimens collected were recorded for each sample. These observations were combined with the Chinese privet observations from the field study 21 June in Montezuma, GA (Macon Co.) on to give a survey of arthropods inhabiting Chinese privet.

Egg parasitoid detection. Sampling for egg parasitoids of *L. mutica* was done by deploying sentinel plants containing *L. mutica* eggs into two test sites with established

lace bug populations: the State Botanical Garden in Clarke Co., GA and a location in Spalding Co., GA (N33° 11.6638', W084° 8.7993'). Twenty-five lace bugs were caged (sewn mesh sleeves, Bridal Inspirations white organza, 664-7242) on designated limbs of potted Chinese privet and green ash (10 plants each) and allowed to oviposit for 24 hours. Lace bugs and cages were removed after 24 hours, and plants were distributed among test sites for 7 days. There were control limbs that cages were placed back over for each test set. This procedure was done at the Spalding Co. site on 22 May and at the Clarke Co. site on 30 May 2013. After 7 days, plants were returned to a greenhouse at the University of Georgia Griffin Campus and cages were placed over the limbs containing lace bug eggs. Plants were evaluated for parasitoid emergence and limbs were examined under the microscope for evidence of parasitism. Lace bug numbers were counted on exposed limbs and control limbs.

Additional efforts to catch parasitoids were made by caging green ash and Chinese privet limbs (forty total limbs at each site) that had evidence of lace bug activity at the State Botanical Garden and Montezuma Bluff Wildlife Management area sites. Limbs were removed a week later and examined for parasitoid emergence.

Statistical analysis. Field study observations of natural enemies on different plant species. Natural enemy counts were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure, and means were separated with Fisher projected least significant difference (LSD) test (SAS Institute 2010).

Field survey of arthropods on *Ligustrum sinense*. Arthropod counts were subjected to a one- way analysis of variance (ANOVA) using the general linear model (GLM)

procedure. The means procedure was used to obtain sums, minimums, and maximums (SAS Institute 2010).

Results

Field study observations of natural enemies on different plant species. Predators.

Beat samples at Montezuma Bluff Wildlife management Area (Macon Co., GA) on 21 June 2013 revealed more crickets (Orthoptera: Gryllidae: *Oecanthus*, *Gryllus fultoni* Alexander,) on swamp privet than the other plant hosts ($F_{2,77} = 9.66$, $P = 0.0002$; Table 4.1). There were more predatory mites (Acari: Phytoseiidae) on green ash and swamp privet than Chinese privet ($F_{2,77} = 6.14$, $P = 0.003$). Carpenter ants (Hymenoptera: Formicidae; *Camponotus*) and predatory thrips (Thysanoptera: Aeolothripidae) were found most often on green ash ($F_{2,77} = 13.62$, $P = 0.0001$; $F_{2,77} = 4.46$, $P = 0.015$). There were no significant differences on plant hosts among spiders (Araneae: Thomisidae, Salticidae, Araneidae) ($F_{2,77} = 2.85$, $P = 0.064$), wasps (Hymenoptera: parasitic) ($F_{2,77} = 0.15$, $P = 0.851$), assassin bugs (Heteroptera: Reduviidae: *Zelus renardii* Kolenati) ($F_{2,77} = 1.93$, $P = 0.153$), or lacewing larvae (Neuroptera: Chrysopidae: *Chrysopa*) ($F_{2,77} = 1.68$, $P = 0.193$). Overall, predators were least found on Chinese privet ($F_{2,78} = 8.01$, $P = 0.0007$).

Beat samples from the State Botanical Gardens (Clarke Co., GA) on 12 June 2013 had more spiders, wasp and tree crickets on Chinese privet than green ash ($F_{1,39} = 6.14$, $P = 0.018$; $F_{1,39} = 4.33$, $P = 0.044$; $F_{1,39} = 10.58$, $P = 0.002$; Table 4.3). Predatory mites ($F_{1,39} = 0.33$, $P = 0.570$), carpenter ants ($F_{1,39} = 0.20$, $P = 0.660$), assassin bugs ($F_{1,39} = 1.05$, $P = 0.435$), predatory thrips ($F_{1,39} = 0.00$, $P = 1.00$) and lacewing larvae ($F_{1,39} = 0$, $P =$

= 0) were found in similar numbers on the two host plants. Overall predators were more common on Chinese privet than green ash ($F_{1,39} = 15.74$, $P = 0.0003$).

Beat sampling from Montezuma Bluff Wildlife Management Area (Macon Co., GA) on 12 June 2013 showed more spiders on swamp privet than other plant hosts ($F_{2,78} = 4.17$, $P = 0.019$; Table 4.2). There were no significant differences on plant hosts for wasps ($F_{2,78} = 0.49$, $P = 0.612$), predatory mites ($F_{2,78} = 1.25$, $P = 0.292$), carpenter ants ($F_{2,78} = 0.56$, $P = 0.575$), assassin bugs ($F_{2,78} = 0.49$, $P = 0.612$), predatory thrips ($F_{2,78} = 1.41$, $P = 0.249$), lacewing larvae ($F_{2,78} = 0.49$, $P = 0.612$), crickets ($F_{2,78} = 1.78$, $P = 0.175$), or overall predators ($F_{2,78} = 0.23$, $P = 0.934$).

A comparison of beat samples collected over alcohol from the Clarke Co. (12 June 2013) and Macon Co. (18 June 2013) locations showed no differences among predators except for more wasp at the Clarke Co. location ($F_{1,157} = 4.44$, $P = 0.036$; Table 4.4). Overall the total predators were equal among predators ($F_{1,118} = 1.24$, $P = 0.267$).

Prey. Beat samples from Montezuma Bluff Wildlife management Area (Macon Co., GA) collected on 21 June 2013 had more beetles (Coleoptera: Scarabidae, Curculionidae) mostly on swamp privet ($F_{2,77} = 4.60$, $P = 0.013$; Table 4.1). Lace bug adults (Hemiptera: Tingidae: *L. mutica*) were predominately on green ash ($F_{2,77} = 5.53$, $P = 0.0057$). There were no significant differences among plant hosts for bark lice (Psocoptera: Psocidae) ($F_{2,77} = 2.09$, $P = 0.131$), plant/leaf plant/ leaf hoppers (Hemiptera: Flattidae, Cicadellidae: *Graphocephala coccinea* Forster, *Anormemis chloris* Melichar) ($F_{2,77} = 1.93$, $P = 0.153$), or overall prey ($F_{2,78} = 1.68$, $P = 0.193$).

Samples from the State Botanical Gardens (Clarke Co., GA) 12 June 2013 had more lace bug adults on green ash ($F_{1,39} = 6.99$, $P = 0.012$; Table 4.3), but the numbers of

plant/leaf hoppers ($F_{1,39} = 3.82$, $P = 0.058$), springtails (Collembola: Isotomidae) ($F_{1,39} = 1.00$, $P = 0.324$), bark lice ($F_{1,39} = 3.03$, $P = 0.090$), whiteflies (Hemiptera: Aleyrodidae) ($F_{1,39} = 1.64$, $P = 0.208$), flies (Diptera: Dolichopidae, Drosophilidae, Muscidae, Caliphoridae) ($F_{1,39} = 0.00$, $P = 1.00$), beetles ($F_{1,39} = 0.11$, $P = 0.744$), incidentals ($F_{1,39} = 0.61$, $P = 0.438$) and overall prey was similar on both hosts ($F_{1,39} = 2.46$, $P = 0.125$).

Samples from Montezuma Bluff Wildlife management Area (Macon Co., GA) on 18 June 2013 had more springtails on Chinese privet ($F_{2,77} = 3.74$, $P = 0.028$; Table 4.2). There were more incidentals (Diptera; Hemiptera: Psyllidae) on swamp privet and Chinese privet ($F_{2,77} = 6.17$, $P = 0.0033$). Lace bugs were found mostly on swamp privet ($F_{2,78} = 4.41$, $P = 0.015$). There were no differences among plant hosts in abundance of plant/leaf hoppers ($F_{2,77} = 0.98$, $P = 0.134$), bark lice ($F_{2,77} = 0.80$, $P = 0.454$), whiteflies ($F_{2,77} = 1.00$, $P = 0.373$), flies ($F_{2,77} = 2.09$, $P = 0.130$), beetles ($F_{2,77} = 1.17$, $P = 0.315$), or total prey ($F_{2,78} = 2.24$, $P = 0.114$).

Samples from Clarke Co. (12 June 2013) and Macon Co. (18 June 2013) had no significant differences among prey except for a greater amount of whiteflies, springtails and lace bugs at the Clarke Co. location ($F_{1,157} = 54.13$, $P = 0.0001$; $F_{1,157} = 3.99$, $P = 0.016$; $F_{1,157} = 4.29$, $P = 0.041$; Table 4.4). Total prey was greatest at the Clarke Co. location ($F_{1,118} = 15.28$, $P = 0.0002$).

Field survey of arthropods on *Ligustrum sinense*. Carpenter ants were the most abundant arthropods on Chinese privet with a total of 327 collected in 358 samples (Table 4.5). Ten ants was the maximum collected in one sample. Spiders (Araneae: Thomisidae, Salticidae, Araneidae) (311 total) and plant/ leaf hoppers (Hemiptera: Flattidae, Cicadellidae: *Graphocephala coccinea* Forster, *Anormemis chloris* Melichar)

(233 total) were the two other abundant arthropods sampled. The total predator to prey ratio was 716:263 for the samples combined.

Egg parasitoid detection. The mean number of lace bugs collected from Chinese privet limbs exposed to predators at the Spalding County site on 22 May 2013 was 2.4 and the control limbs number was 5 (Table 4.6). Green ash had a mean number of lace bugs of 10.1 and control number of 16. The mean number of lace bugs collected from exposed Chinese privet limbs at the Clarke County site on 30 May 2013 was 4.3 and the control limb number was 7. Green ash had a mean number of lace bugs of 6.7 and control number of 9. One mymarid wasp (Hymenoptera) was recovered from green ash in Clarke Co. No parasitoids were recovered from the additional efforts of caged limbs at the State Botanical Garden and Montezuma Bluff Wildlife Management area sites.

Discussion

Our observations of predators on the different plant species do not support the hypothesis that the natural enemy complex is preventing the *L. mutica* from establishing on Chinese privet. Except for crickets, Chinese privet never had more predators than other plant species. More wasps were found on Chinese privet at the Clarke Co. location. Plant/leaf hoppers (Hemiptera) were one of the most common plant feeders on all the plant species. Springtails (Collembola) were more common on Chinese privet in Clarke Co., which could be due in part to fewer predators inhabiting the Chinese privet. Spiders were a common predator among all plant species, and are known to attack other lace bug species *S. pyrioides* Scott, the azalea lace bug (Shrewsbury et al. 2004).

L. mutica did seem to favor the green ash with some preference to swamp privet except on the 18 June 2013 sampling at the Montezuma Bluff Wildlife Management

Area. On this date, the lace bugs were concentrated more on the swamp privet. This could be in part due to environmental conditions. In the swamp, most of the Chinese privet and green ash are seedlings no more than a meter tall. The swamp privet is more commonly found as established trees. The week previous to sampling the river near the site flooded and the seedling Chinese privet and green ash were all under water for several days. Lace bugs may have moved to the higher limbs of swamp privet during this time.

The egg parasitoid results revealed evidence of some parasitoid activity on eggs with the finding of a mymarid wasp. Although only one was found, there is potential for there to be more by having missed some due to their incredibly minute size (size of pen tip). Also, their developmental time is not known and may take longer than when the samples were examined. The exposed limb sample numbers were lower than the control sample numbers indicating some type of predation on eggs. In one case, a minute pirate bug (Hemiptera: Anthocoridae) was found trapped in the cage with a sentinel limb and the sample results had no lace bug emergence.

Dry sampling versus wet sampling was done in hopes to catch more specimens and be able to better identify them. The dry samples were beneficial in that results could be recorded immediately and seemed to be accurate in observing most specimens. The wet sampling, did however allow for some smaller specimens to be detected such as collembola, small parasitic wasp, and midges. This was mainly important in the observation of the wasps.

The survey of arthropods on Chinese privet was done primarily to give an idea of what was on Chinese privet and in what kind of densities at a given time. Ants and spiders tend to be the most abundant predators, and plant/leaf hoppers consistently seem

to be the most abundant plant feeders. Chinese privet in combination with observations of the other field trials does not seem to ever be heavily infested with predators or prey. The predator to prey ratio from the survey produced a predator/ prey ratio of 716: 263, which suggest that predators may keep the prey on plants reduced.

From these results, if released, *L. hospita* should be able to effectively colonize the Chinese privet. There are generalist predators, but they often are not found as commonly on the Chinese privet as other plant host where the native lace bug occurs. The only specific *Leptoypha* spp. natural enemy found was the mymarid wasp, but at this point it cannot be concluded as a limiting factor for *L. hospita*'s success.

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Table 4.1. Comparison assay for mean \pm S.E. of predators/prey on plant species green ash, Chinese privet and swamp privet from beat samples at Montezuma Bluff Wildlife management Area (Macon Co., GA) on 21 June 2012.

	Green ash	Chinese privet	Swamp privet
<i>Predators</i>			
Spider	1.07 \pm 0.10 B	1.07 \pm 0.20 B	1.70 \pm 0.30 A
Wasp	0.08 \pm 0.10 A	0.05 \pm 0.03 A	0.05 \pm 0.03 A
Predatory mite	0.45 \pm 0.10 A	0.03 \pm 0.30 B	0.33 \pm 0.09 A
Carpenter ant	0.90 \pm 0.20 A	0.13 \pm 0.05 B	0.05 \pm 0.05 B
Assassin bug	0.08 \pm 0.04 A	0.00 \pm 0.00 A	0.13 \pm 0.06 A
Predatory thrips	1.45 \pm 0.10 A	0.08 \pm 0.04 B	0.28 \pm 0.10 AB
Lacewing larvae	0.03 \pm 0.03 A	0.05 \pm 0.04 A	0.23 \pm 0.10 A
Cricket	0.00 \pm 0.00 B	0.23 \pm 0.09 B	0.60 \pm 0.20 A
Total predators	3.05 \pm 0.34 A	3.35 \pm 0.44 A	1.68 \pm 0.22 B
<i>Prey</i>			
Beetle	0.25 \pm 0.03 B	0.05 \pm 0.04 B	0.20 \pm 0.10 A
Plant/ leaf hopper	0.33 \pm 0.10 A	1.05 \pm 0.30 A	0.95 \pm 0.30 A
Bark louse	0.05 \pm 0.03 A	0.13 \pm 0.10 A	0.23 \pm 0.10 A
Lace bug	0.73 \pm 0.19 A	0.10 \pm 0.08 B	0.45 \pm 0.14 AB
Total prey	1.12 \pm 0.26 A	1.82 \pm 0.31 A	1.28 \pm 0.26 A

Means in the in the same row bearing different letters are significantly different ($\alpha = 0.05$).

Table 4.2. Comparison assay for predators/prey on plant species green ash, swamp privet, and Chinese privet from beat samples with alcohol at State Botanical Gardens (Clarke Co., GA) 12 June 2013.

	Green ash	Chinese privet
<i>Predators</i>		
Spider	0.13 ± 0.06 B	0.48 ± 0.12 A
Wasp	0.03 ± 0.03 B	0.13 ± 0.05 A
Predatory mite	0.03 ± 0.03 A	0.05 ± 0.03 A
Carpenter ant	0.08 ± 0.04 A	0.05 ± 0.03 A
Assassin bug	0.00 ± 0.00 A	0.08 ± 0.04 A
Predatory thrips	0.03 ± 0.03 A	0.03 ± 0.03 A
Lacewing larvae	0.00 ± 0.00 A	0.00 ± 0.00 A
Cricket	0.03 ± 0.03 B	0.43 ± 0.12 A
Total predators	0.30 ± 0.10 B	1.23 ± 0.22 A
<i>Prey</i>		
Plant/ leaf hopper	0.50 ± 0.13 A	0.88 ± 0.16 A
Bark louse	0.10 ± 0.06 A	0.28 ± 0.09 A
Whitefly	0.53 ± 0.15 A	0.76 ± 0.16 A
Fly	0.08 ± 0.04 A	0.08 ± 0.04 A
Springtail	0.13 ± 0.05 A	0.23 ± 0.11 A
Beetle	0.01 ± 0.05 A	0.13 ± 0.05 A
Lace bug	0.48 ± 0.13 A	0.10 ± 0.05 B
Incidentals	0.13 ± 0.06 A	0.03 ± 0.03 A
Total prey	2.38 ± 0.29 A	3.08 ± 0.34 A

Means in the in the same row bearing different letters are significantly different ($\alpha = 0.05$).

Table 4.3. Comparison assay for mean \pm S.E. of predators/prey on plant species green ash, Chinese privet, and swamp privet from beat samples with alcohol at Montezuma Bluff Wildlife Management Area (Macon Co., GA) 18 June 2013.

	Green ash	Chinese privet	Swamp privet
<i>Predators</i>			
Spider	0.23 \pm 0.10 B	0.35 \pm 0.10 B	0.67 \pm 0.20 A
Wasp	0.03 \pm 0.03 A	0.00 \pm 0.00 A	0.03 \pm 0.03 A
Predatory mite	0.23 \pm 0.10 A	0.38 \pm 0.20 A	0.08 \pm 0.04 A
Carpenter ant	0.30 \pm 0.10 A	0.18 \pm 0.10 A	0.15 \pm 0.08 A
Assassin bug	0.25 \pm 0.03 A	0.25 \pm 0.03 A	0.00 \pm 0.00 A
Predatory thrips	0.75 \pm 0.04 A	0.00 \pm 0.00 A	0.05 \pm 0.03 A
Lacewing larvae	0.03 \pm 0.03 A	0.03 \pm 0.03 A	0.00 \pm 0.00 A
Cricket	0.05 \pm 0.03 A	0.10 \pm 0.05 A	0.20 \pm 0.08 A
Total predators	0.95 \pm 0.21 A	1.05 \pm 0.25 A	1.18 \pm 0.20 A
<i>Prey</i>			
Plant/ leaf hopper	0.48 \pm 0.20 A	0.75 \pm 0.20 A	0.30 \pm 0.11A
Bark louse	0.08 \pm 0.04 A	0.03 \pm 0.03 A	0.13 \pm 0.08 A
Whitefly	0.03 \pm 0.03 A	0.00 \pm 0.00 A	0.00 \pm 0.00 A
Fly	0.28 \pm 0.09 A	0.08 \pm 0.10 A	0.10 \pm 0.10 A
Springtail	0.00 \pm 0.00 B	0.13 \pm 0.10 A	0.03 \pm 0.02 B
Beetle	0.03 \pm 0.03 A	0.05 \pm 0.03 A	0.10 \pm 0.05 A
Lace bug	0.13 \pm 0.05 B	0.10 \pm 0.09 B	0.45 \pm 0.13 A
Incidentals	0.18 \pm 0.10 B	0.08 \pm 0.08 A	0.00 \pm 0.00 A
Total prey	1.18 \pm 0.30 A	1.95 \pm 0.27 A	1.88 \pm 0.30 A

Means in the in the same row bearing different letters are significantly different ($\alpha = 0.05$).

Table 4.4. Comparison assay for predators/prey from beat samples with alcohol at State Botanical Gardens (Clarke Co., GA) 12 June 2013 and Montezuma Bluff Wildlife Management Area (Macon Co., GA) 18 June 2013.

	Clarke Co.	Macon Co.
<i>Predators</i>		
Spider	0.30 ± 0.07 A	0.42 ± 0.07 A
Wasp	0.08 ± 0.03 A	0.02 ± 0.01 B
Predatory mite	0.04 ± 0.02 A	0.23 ± 0.08 A
Carpenter ant	0.06 ± 0.03 A	0.21 ± 0.06 A
Assassin bug	0.04 ± 0.02 A	0.02 ± 0.01 A
Predatory thrips	0.03 ± 0.02 A	0.04 ± 0.02 A
Lacewing larvae	0.00 ± 0.00 A	0.02 ± 0.01 A
Cricket	0.22 ± 0.07 A	0.12 ± 0.03 A
Total predators	0.76 ± 0.13 A	1.00 ± 0.17 A
<i>Prey</i>		
Plant/ leaf hopper	0.70 ± 0.11 A	0.50 ± 0.09 A
Bark louse	0.19 ± 0.05 A	0.08 ± 0.03 A
Whitefly	0.65 ± 0.11 A	0.01 ± 0.01 B
Fly	0.08 ± 0.03 A	0.15 ± 0.04 A
Springtail	0.18 ± 0.06 A	0.05 ± 0.02 B
Beetle	0.11 ± 0.04 A	0.06 ± 0.02 A
Lace bug	0.29 ± 0.07 A	0.11 ± 0.05 B
Incidentals	0.08 ± 0.03 A	0.04 ± 0.03 A
Total prey	2.73 ± 0.22 A	1.56 ± 0.21 B

Means in the in the same row bearing different letters are significantly different ($\alpha = 0.05$).

Table 4.5. Survey of arthropods inhabiting Chinese privet observed from field beat samples.

Arthropods	N	Sum	Min	Max
Carpenter ants	358	327	0	10
Spiders	358	311	0	7
Plant/ leaf hoppers	358	233	0	7
Predatory thrips	358	37	0	3
Beetles	358	30	0	2
Wasp	358	18	0	1
Cricket	358	17	0	3
Lacewing larvae	358	6	0	1
<i>Total Predators</i>	716			
<i>Total Prey</i>	263			

N= number of observations per arthropod; min & max values based on individual observations.

Table 4.6. Mean number of lace bugs on exposed limbs and numbers on control limbs from egg parasitism tests.

Plant type/ location	Exposed limbs	Control limbs
Chinese privet (Spalding Co.)	2.4	5
Green ash (Spalding Co.)	10.1	16
Chinese privet (Clarke Co.)	4.3	7
Green ash (Clarke Co.)	6.7	9

CHAPTER 5
CONCLUSION

The series of studies reported herein have further elucidated the lace bug *Leptoypha mutica* Say and its relationship with Chinese privet, *Ligustrum sinense* Lour, as well as, provided information for the lace bug *Leptoypha hospita* Drake et Poor's potential release. The main goals of these studies were to determine *L. mutica*'s ability to utilize Chinese privet, determine its host preference, evaluate its development biology on Chinese privet, and define natural enemy complexes associated with Chinese privet.

Original collections and observations of the two lace bug populations suggested they could have been two different lace bug species. The lace bugs collected from swamp privet (Macon Co.) appeared slightly smaller in size and more mobile than those collected off of fringe trees (Spalding Co.). However, the two lace bug populations used in this study were identified in February 2012 as *L. mutica* (personal communication Tom Henry USDA/ARS/SEL) and they behaved similarly throughout the studies. The no-choice test general comparison of the two lace bug populations showed no differences in their performance except for more egg production by population 1. This, however, was probably a direct result of their higher survival in that test. The laboratory host choice test also supports their identification as one species in that there were no differences between the populations and their host choice.

Prior to this study, Chinese privet was not a confirmed host of *L. mutica*, although it was known to utilize other members of the Oleaceae family including fringe tree, *Chionanthus virginicus* L., green ash, *Fraxinus pennsylvanica* Marsh, and swamp privet, *Foresteria acuminata* Michx.,. The no choice test showed that *L. mutica* adults could survive, feed, and lay eggs on Chinese privet. Results did indicate less egg production on Chinese privet. This could mean that lace bugs do not obtain the same amount of energy

from Chinese privet, so it was less acceptable or simply the lace bugs used were unable to produce eggs. Also, observations of egg placement on the different plant hosts showed occasional exposed eggs on the Chinese privet, whereas egg placement on other hosts was deeper in the plant tissue (unpublished data). These observations could be clues to as why Chinese privet is not a preferred host plant.

In a small trial 5 male and 5 female lace bugs were caged with Chinese privet from 14 December 2011 to 29 March 2012 at which time there were over 100 adults (unpublished data). Therefore, the lace bugs have the potential to utilize Chinese privet, but we have never observed significant infestation in the wild on Chinese privet. Our studies suggest that the reason *L. mutica* does not readily utilize Chinese privet is because it is not a preferred host. When given only the choice of swamp privet and Chinese privet, the lace bug did not prefer one to the other, but whenever green ash was present the lace bugs always preferred it. In most forest areas where *L. mutica* were established green ash was also present, so it is likely that ash was the primary host supporting the population. This restricted host preference makes *L. mutica* an unlikely candidate for control of Chinese privet without a host shift, but it is encouraging for the similar Asian lace bug, *L. hospita*'s potential as a biological control agent. Chinese privet is the preferred host of this lace bug, and if released, acting similarly, it will not shift to other native plant hosts.

Studying the developmental biology of *L. mutica* is useful not only to better understand the behavior of the lace bug, but also to analyze its full potential as a biological control agent. The lace bugs were able to develop at all temperatures, but were least successful at surviving at 20°C. Other factors could have contributed to mortality other than temperature alone such as damage while transferring first instars. The

regression equations for the reciprocal of developmental times on temperature for each life stage and values for T_0 and K were used to predict at what temperature each life stage would cease to develop, and it was estimated that complete development would cease below 10.5°C. This relatively low threshold and having a broad range over which development occurs, suggests the potential utility of this insect under a variety of environmental conditions. *L. mutica*'s developmental time at 24 – 26°C is \approx 10 days shorter than *L. hospita*'s, which could further support that Chinese privet is potential host for the native lace bug but not an optimal host.

Our observations of predators on the different plant species do not support the hypothesis that the natural enemy complex is preventing the *L. mutica* from establishing on Chinese privet. Except for crickets, Chinese privet never had more predators than other plant species. More wasps were found on Chinese privet at the Clarke Co. location. Plant/leaf hoppers (Hemiptera) were one of the most common plant feeders on all the plant species. Springtails (Collembola) were more common on Chinese privet in Clarke Co., which could be due in part to fewer predators inhabiting the Chinese privet.

The egg parasitoid results revealed evidence of some parasitoid activity on eggs with the finding of a mymarid wasp. Although only one was found, there is potential for there to be more by having missed some due to their incredibly minute size (size of pen tip). Also, their developmental time is not known and may take longer than when the samples were examined. The exposed limb sample numbers were lower than the control sample numbers indicating some type of predation on eggs.

From these results, if released, *L. hospita* should be able to effectively colonize the Chinese privet. There are generalist predators, but they often are not found as

commonly on the Chinese privet as other plant host where the native lace bug occurs. The only specific *Leptoypha* spp. natural enemy found was the mymarid wasp, but at this point it cannot be concluded as a limiting factor for *L. hospita*'s success.