Developmental Biology of *Leptoypha mutica* (Hemiptera: Tingidae) on Chinese Privet (Lamiales: Oleaceae)[1]

J. Kalina[2], S.K. Braman[3], and J.L. Hanula[4]

University of Georgia, Department of Entomology, 413 Biological Sciences Building, Athens, Georgia 30602 USA

J. Entomol. Sci. 52(2): 154–160 (April 2017)

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 its potential to establish on a new host, developmental biology on Chinese privet was studied at temperatures of 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 d. Estimated threshold temperatures and calculated thermal unit requirements for egg development were 11.0°C and 211.9 degree-days (DD); for nymphal development, 9.9°C and 326.8 DD; and for complete development, 10.5°C and 527.4 DD. Results of this study indicate that *L. mutica* will oviposit and can develop successfully on Chinese privet over a range of temperatures suggesting the potential for multiple generations to occur in a single season.

Key Words *Leptoypha mutica*, *Ligustrum sinense*, Chinese privet, developmental biology, 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 range of environments and habitats, Chinese privet has become a widespread exotic plant throughout 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 approximately 7.1 million ha where the privet occurs, it densely covers approximately 1 million ha (Miller et al. 2008, Rudis et al. 2006). In these areas, 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 (Merriam and Feil 2002, Morris et al. 2002, U.S. Fish and Wildlife Service 1992). Other problems are decreased invertebrate communities and toxic effects on humans and animals (Biesmeijer et al. 2006, Crisp et al. 1998, Kerr et al. 1999).

[1]Received 24 June 2016; accepted for publication 28 September 2016.
[2]Present address: Dow AgroSciences, LLC, 4400A Ambassador Caffery, no. 516, Lafayette, LA 70508 USA.
[3]Corresponding author (email: kbraman@uga.edu).
[4]Emeritus, USDA Forest Service, Southern Forest Research Station, 320 Green St., Athens, GA 30602 USA.

Once the privet is established, it is difficult to remove and large-scale removal becomes labor intensive and requires the use of herbicides (Hanula et al. 2009). To help alleviate its 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, a survey of insects feeding on Chinese privet found *Leptoypha hospita* Drake et Poor (Hemiptera: Tingidae) to be a potential biocontrol agent because its common feeding on the privet sometimes resulted in plant dieback (Zhang et al. 2008, 2011). The potential of *L. hospita* as a control agent initiated further examination of the 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 (McFadyen 2003), but only a small proportion of multiple releases may prove to be effective (Denoth et al. 2002, McEvoy and Coombs 2000,Myers 1985). In efforts to prevent futile introductions, the candidate of interest should be extensively evaluated. Thus, the objective of this study was to establish the relationship between temperature and development of *L. mutica* on Chinese privet. Understanding 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* has not already taken advantage of Chinese privet as a host.

Materials and Methods

Leptoypha mutica adults used for this study were collected from fringe trees (*C. virginicus*) in Spalding Co., GA (N 33°11.6638′, W 84°8.7993′), and green ash (*F.pennsylvanica*) and swamp privet (*F. acuminata*) from the Montezuma Bluff Wildlife Management Area (Macon, Co., GA, N 32°20.274′, W 84°1.2245′). Lace bugs were reared in the laboratory on fringe tree leaves in plastic petri dishes at a 14:10 (light:dark) photoperiod at approx. 23°C on the University of Georgia Griffin Campus.

Cuttings of Chinese privet were obtained from potted greenhouse plants grown from field-collected seedlings. Cuttings were placed in cages constructed from 32-ml plastic cups (Jetware PC-100, Bio Serv, Frenchtown, NJ). Cages consisted of a water-filled cup with a lid with a small hole. A piece of Tygon tubing was placed in that hole, and the plant cutting was inserted into the tube and secured with Parafilm wrap (Fisher Scientific, Hampton, NH). Several male and female lace bugs (approximately 10 total) were added to each plant cutting, and another cup with a screen bottom was placed on top and secured with Parafilm. Cages were placed in Percival environmental growth chambers (Model: I-36VL, Percival Scientific, Inc., Perry, IA) at four separate temperatures (± 0.5°C): 20°C, 24°C, 28°C, and 32°C. Females were allowed to oviposit for 24 h at each temperature, then all lace bugs on the cuttings were moved to new cages with fresh cuttings of Chinese privet, and the process was repeated.

To determine the development time of each life stage, all cages were monitored twice a day (10 a.m. and 4 p.m.) under a dissecting microscope for the emergence of nymphs or molted individuals. First instars were transferred individually onto separate caged cuttings of Chinese privet 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 then 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 complete development. The effect of gender on development was compared at each temperature using the *t* test procedure (PROC TTest; SAS Institute 2010).

Least squares linear regression was used to establish the relationship between developmental rate (days) and temperature (°C) over the linear portion of development curves. The lower temperature threshold (T_0) was estimated 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 T_0 value. 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, $K_t = (T - T_0) \times D_t$, where T was 20°C, 24°C, 28°C, or 32°C; T_0 was the lower temperature threshold of a stage of interest and, D_t was the mean development time (days) for a 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. The cephalic pole was characterized by an oval-shaped operculum, which was easily visible when 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 d with decreasing temperatures (Table 1). Less than half of the total development occurred in the egg stage.

There are five nymphal instars of *L. mutica*. The nymphs were oblong and flat. Each instar 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 approximately 1 d. 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 hatching. Their feeding on the mesophyll tissue produced chlorotic, bleached-appearing leaves. Total nymphal development ranged from 14.7 to 33.9 d as temperatures decreased (Table 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 within approximately 1 d. Males could be distinguished by their narrow abdomens, whereas females had a broader abdomen with an ovipositor present. The duration

Table 1. Mean ± SE duration of development in days, and number of individuals completing each stage, of *Leptoypha mutica* on *Ligustrum sinense* cuttings.*

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

* Values in parentheses are numbers of individuals entering each stage.

Table 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 *Leptoypha mutica* (See text for discussion of calculation of T_0 and K).

Developmental Stage	Equation and r^2 *	T_0 (°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.

of complete development to adulthood ranged from 24.4 to 57.1 d with decreasing temperature (Table 1). The lace bugs were able to develop at all temperatures tested, but had the least success at 20°C, with only 7.7% survival ($n = 26$). The optimal temperature for complete development was 28°C, with 52.0% survival ($n = 102$) to the adult stage. Female and male developmental times did not differ significantly at any temperature for any life stage ($\alpha > 0.05$). The male:female sex ratio for 20°C was 1:1; at 24°C it was 1.3:1; at 28°C, 0.9:1; and at 32°C, 1.1:1.

The T_0 value was 11.0°C for egg development, 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_t) 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 DD for fifth instars, 326.8 DD for total nymphal development, and 527.4 DD for complete development. Backward extrapolation of the linear portion of the temperature/development curves predicted the threshold values (Table 2; Fig. 1).

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 tested, but were least successful at surviving at 20°C; however, other factors could have contributed to mortality (i.e., handling). The regression equations for the reciprocal of develop-

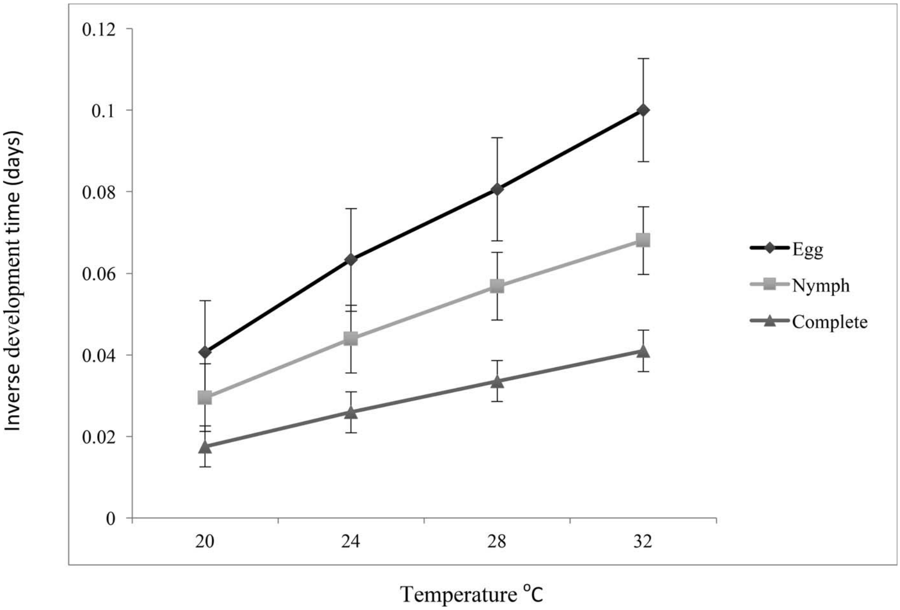

Fig. 1. Prediction of developmental thresholds based on inverse developmental means for time (in days) for egg to hatch, for total nymphal development, and for complete development.

mental 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 occurs from Texas to Florida and northward to the New England states (USDA–Natural Resources Conservation Service 2002). *Leptoypha mutica* is found in a similar range, having been reported from Maine, west to North Dakota, and south to Florida and Texas (Mead 1975).

Development of the Asian lace bug, *L. hospita*, at 24–26°C indicates total development occurred in 24.77 d on Chinese privet (Zhang et al. 2011) in comparison to the *L. mutica* at 34.2 d 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 overwinters as an adult. If a host shift occurred, their relatively short generation time and multiple broods would aid in their ability to potentially colonize Chinese privet.

References Cited

Barber, H.G. and H.B. Weiss. 1922. The lace bugs of New Jersey. State of New Jersey Dept. Agric., Bur. Stat. Insp. Circ. 54: 3–15.

Biesmeijer, J.C., S. Roberts, M. Reemer, R. Ohlemüller, M. Edwards, T. Peters, A. Schaffers, S. Potts, R. Kleukers and C. Thomas. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313: 351–354.

Brown, C.E. and S.R. Pezeshki. 2000. A study on waterlogging as a potential tool to control *Ligustrum sinense* populations in western Tennessee. Wetlands 20: 429–437.

Crisp, P.N., K. Dickinson and G. Gibbs. 1998. Does native invertebrate diversity reflect native plant diversity? A case study from New Zealand and implications for conservation. Biol. Cons. 83: 209–220.

Denoth, M., L. Frid and J.H. Myers. 2002. Multiple agents in biological control: Improving the odds? Biol. Control 24: 20–30.

Dirr, M.A. 1983. Manual of Woody Landscape Plants. 3rd ed. Stipes Publishing Co., Champaign, IL. 536 pp.

Drake, C.J. 1918. Notes on North American Tingidae. Bull. Brooklyn Entomol. Soc. 13: 86–88.

Hanula, J.L., S. Horn and J.W. Taylor. 2009. Chinese privet (*Ligustrum sinense*) removal and its effect on native plant communities of riparian forests. Invasive Plant Sci. Manag. 2: 292–300.

Kerr, L., A. Kelch and J. William. 1999. Fatal privet (*Ligustrum amurease*) toxicosis in Tennessee cows. Vet. Hum. Toxicol. 41: 391–392.

McEvoy, P.B. and E.M. Coombs. 2000. Why Things Bite Back: Unintended Consequences of Biological Weed Control. Kluwer Academic Publishers, Boston, MA. Pp. 167–194.

McFadyen, R.E. 2003. Does ecology help in the selection of biocontrol agents? Glen Osmond, Australia. Coop. Res. Centre Aust. Weed Manag. 7: 5–9.

Mead, F.W. 1975. The fringe tree lace bug, *Leptoypha mutica*. Florida Dept. of Agric. Consu. Serv. Div. Plant Ind., Gainesville. Entomol. Circ. No. 161.

Merriam, R.W. and E. Feil. 2002. The potential impact of an introduced shrub on native plant diversity and forest regeneration. Biol. Invasions 4: 369–373.

Miller, J.H., E.B. Chambliss and C.M. Oswalt. 2008. Maps of occupation and estimates of acres covered by nonnative invasive plants in southern forest using SRS FIA data posted on March 15, 2008. http://www.srs.fs.usda.pubs.ja.ja_gan007.pdf.

Morris L.L., J.L. Walck and S.N. Hidayati. 2002. Growth and reproduction of the invasive *Ligustrum sinense* and native *Forestiera ligustrina* (Oleacea): Implications for the invasion and persistence of a nonnative shrub. Int. J. Plant Sci. 163: 1001–1010.

Myers, J.H. 1985. How many insects are necessary for successful biocontrol of weeds? Pp. 77–82. *In* Proc. VI Int. Symp. Biol. Contr. Weeds. Agriculture Canada, Ottawa, Vancouver, Canada.

Rudis, V.A., A. Gray, W. McWilliams, R. O'Brien, C. Olson, S. Oswalt and B. Schulz. 2006. Regional monitoring of nonnative plant invasions with the Forest Inventory and Analysis program, Pp. 49–64 *In* McRoberts, R.E. and G. A. Reams (eds.), Proc. Sixth Annual Forest Inventory Analysis Symposium, 2004 September 21–24, Denver, CO. Gen. Tech. Rep. WO-70. U.S. Department of Agriculture, Forest Service, Washington DC.

SAS Institute. 2010. SAS Guide for Personal Computers. 6th ed. SAS Institute, Cary, NC.

Small, J.K. 1933. Manual of the southeastern flora, part one and two. Univ. North Carolina Press, Chapel Hill. 1554 pp.

USDA–Natural Resources Conservation Service. 2002. The PLANTS Database, Version 3.5. (http://plants.usda .goc/, accessed 15 June 2016).

U.S. Fish and Wildlife Service. 1992. Endangered and Threatened Species of the Southeastern United States (The Red Book). U.S. Fish and Wildlife Service, Atlanta, GA.

Zhang, Y., J.L. Hanula, S. Horn, S.K. Braman and J. Sun. 2011. Biology of *Leptoypha hospita* (Hemiptera: Tingidae), a potential biological control agent of Chinese privet. Ann. Entomol. Soc. Am. 104: 1327–1333.

Zhang, Y.Z., J.L. Hanula and J.H. Sun. 2008. Survey for potential insect biological control agents of *Ligustrum sinense* (Scrophulariales: Oleaceae) in China. Fla. Entomol. 91: 372–382.