

# Biology and Life History of *Argopistes tsekooni* (Coleoptera: Chrysomelidae) in China, a Promising Biological Control Agent of Chinese Privet

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Ann. Entomol. Soc. Am. 102(3): 508–516 (2009)

**ABSTRACT** The biology and life history of *Argopistes tsekooni* Chen (Coleoptera: Chrysomelidae), a potential biological control agent of Chinese privet, *Ligustrum sinense* Lour., was studied under laboratory and outdoor conditions in Huangshan City of Anhui Province, China, in 2006. *A. tsekooni* larvae are leafminers that undergo three instars. The first two instars feed exclusively within the leaf. The third instar feeds within the leaf until larval development is complete and then falls to the ground where it burrows into the soil to pupate. Larval development is completed within a single mine. Adult *A. tsekooni* is also a foliage feeder, consuming  $8.32 \pm 0.96$  mm<sup>2</sup> of privet leaf per day. A female lays an average of  $27.8 \pm 5.4$  eggs within 30 d. *A. tsekooni* overwinters as an adult and had three overlapping generations in Anhui Province, an area similar in climate to Atlanta, GA. Biological attributes of *A. tsekooni*, such as high damage levels caused by larvae and adults and multiple overlapping generations per year, suggest that this flea beetle is a good candidate for biological control of Chinese privet in the United States.

**KEY WORDS** life history, *Argopistes tsekooni*, *Ligustrum sinense*, biological control

Chinese privet, *Ligustrum sinense* Lour. (Oleaceae), is a semideciduous shade-tolerant shrub or small tree native to China (Miller 2003). It is a rapidly encroaching plant that continues to invade disturbed sites, fencerows, and bottomland and upland forests in the Southeastern United States (Dirr 1998, Haragan 1996, Miller 2003). Chinese privet matures rapidly and produces seeds in large numbers that are spread mainly by wildlife, especially birds (Swarbrick et al. 1999, White and Stiles 1992). Once liberated from their fleshy fruit, privet seeds germinate promptly without cold stratification (Burrows and Kohen 1986, Young and Young 1992). Chinese privet also reproduces by means of root suckers. Because of the species' shade tolerance and abundant regeneration, privet is able to spread and thrive under dense forest canopies where it is an important factor limiting hardwood regeneration, wildlife habitat, biodiversity, and recreational activities in heavily infested areas (Harrington and Miller 2005).

Introduced from China in 1852 as an ornamental shrub (Dirr 1998), Chinese privet has escaped from cultivation and naturalized itself in 19 states east of the

Rockies, ranging from Massachusetts to Florida, and west to Texas (Ward 2002). After rapidly expanding at an exponential rate from 1950 to 1980, Chinese privet is now present in >40% of southeastern U.S. counties (USDA-NRCS 2003), and it is ranked among the top 10 exotic plant pests of Georgia (Georgia Exotic Pest Plant Council 2003) and Mississippi (Matlack 2002). Large-scale control of privet is labor-intensive and requires the use of large amounts of herbicides, so biological control offers the most cost effective and the best long-term control option.

Surveys for potential biological control agents were conducted in China during 2005 and 2006. More than 100 phytophagous insect species were found feeding on Chinese privet (Zhang et al. 2008a), of which the most promising insect for biological control was the flea beetle *Argopistes tsekooni* Chen (Coleoptera: Chrysomelidae). Larvae of this beetle feed within the mesophyll tissue of the leaf, causing mines, whereas adults feed on the leaves, causing small holes (Yu et al. 1996, Inoue 2006). A preliminary host specificity study of *A. tsekooni* conducted in China showed that the flea beetle had a very narrow host range. It preferred feeding and ovipositing on *Ligustrum* spp. (Zhang et al. 2008b), most of which are invasive in the United States. The flea beetle can cause mortality of naturally occurring Chinese privet in China when its population is high, and it is the dominant species in the insect community feeding on this plant (unpublished data).

Detailed biological and ecological studies of *A. tsekooni* are surprisingly few despite the extensive defo-

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liaison caused by the larval and adult stages feeding on ornamental privet in China (Yu et al., 2005). To evaluate its potential as a biological control of Chinese privet, knowledge of the biology and life history of *A. tsekooni* is important. Biological aspects of *A. tsekooni* were therefore investigated in a shaded outdoor site and under natural conditions in China.

### Materials and Methods

**Study Site.** Biology and life history studies of *A. tsekooni* were conducted in the laboratory and field in Huangshan City, which lies in the south of Anhui Province. Huangshan City is situated in the northern part of the mid-subtropical zone ( $29^{\circ} 24' - 30^{\circ} 31' N$ ,  $117^{\circ} 12' - 118^{\circ} 53' E$ ) and belongs to the subtropical monsoon moist climatic zone. The field study site was located at  $29^{\circ} 43' N$ ,  $118^{\circ} 16' E$  in Huangshan City, at 200-m elevation. The average annual temperature of Huangshan City is  $15.4 - 16.8^{\circ}C$ , and the average temperature in July and January is  $28.6$  and  $4.9^{\circ}C$ , respectively. Average annual precipitation is 1,295–1,702 mm, annual mean relative humidity is 78%, and the frost-free period is  $\approx 230$  d (Zhang and Yang 2007). Huangshan City belongs to the subtropical evergreen broadleaved forest vegetation zone, as well as the mountain evergreen broadleaf forest and deciduous broadleaved forest zones (Zhang and Yang 2007). The area has been significantly altered by human activities, and only nature reserves and forest parks contain relatively undisturbed forest communities. The main plant species are *Pinus massoniana* Lamb., *Pinus taiwanensis* Hayata, *Cunninghamia lanceolata* (Lamb.) Hook, and *Phyllostachys edulis* (Carr.) H. de Lehaie (unpublished data).

**Insect and Plant Source.** Adults of *A. tsekooni* used in laboratory and outdoor studies were collected from Chinese privet at field sites in Huangshan City. Overwintered adults were collected 10 April and used for biology studies of the first generation. Newly eclosed adults of the first generation were collected 8 June for studies of the second generation and newly eclosed adults of the second generation were collected 2 August for studies of the third generation. Newly eclosed adults are yellowish brown and easily separated from older adults. We determined beetle generations based on the apparent peaks in numbers of eggs and larvae.

Field-collected adults were separated by sex and placed on 3-yr-old potted *L. sinense* saplings in columniform cages made of fine polyester organza over an iron wire frame (20 cm in diameter and 60 cm in height). A twist tie was used to hold the top of the cage closed. Adults were sexed by assessing the shape of the terminal abdominal sternite (Yu et al. 1996; Fig. 1E and F). The caged flea beetles provided sufficient insects for our experiments below. All potted Chinese privets used in the tests were held under natural day lengths and temperatures in an outdoor site beneath a shade cloth supported on poles. Plants were irrigated as needed, but no pesticides or fertilizers were used to avoid any effect they might have on *A. tsekooni* biology or behavior.

**Laboratory and Outdoor Studies.** Studies of the duration and characterization of life stages in each generation of *A. tsekooni* were conducted in the insect laboratory of the Forestry Institute of Huangshan City and the shaded outdoor site mentioned above. The experiments were conducted from early April to mid-September. The average temperature and relative humidity during each experimental period are reported in Table 1 (data were obtained from China Meteorological Data Sharing Service System, <http://cdc.cma.gov.cn/>).

**Egg.** In the outdoor study area, 10 pairs of adults were caged individually (one male and one female per cage) on branches of potted Chinese privet enclosed in polyester organza sleeve bags (10 by 20 cm). To precisely determine the exact time of oviposition, adult pairs were moved with their sleeve bag to another fresh branch every 12 h. Leaves on the previous branch were checked carefully for eggs using a stereomicroscope to determine when oviposition occurred. Duration of the egg stage was determined by observing newly laid eggs twice a day until they hatched. This was done for each of the three generations in mid-April (average temperature  $17.1^{\circ}C$ ), mid-June (average temperature  $26.9^{\circ}C$ ), and early August (average temperature,  $29.4^{\circ}C$ ), respectively. In total, 50, 80, and 81 eggs were observed for the three generations, respectively. The size of eggs was determined by measuring the length and width of 53 eggs with a calibrated ocular micrometer in a stereomicroscope.

**Larva and Pupa.** Duration of the larval stage was determined by observing infested leaves after egg hatch. Duration of larval development was measured for 42, 65, and 50 larvae during the first, second, and third generations, respectively. A 3-cm-deep, 20-cm-diameter petri dish full of disinfected moist fine sand was provided at the bottom of the potted plants, just under leaves with larvae, so mature larvae dropping to the soil to pupate could be collected and observed. The number of larval instars was determined by counting and measuring head capsules, dissected from mines with a special needle and forceps, before and after mature larvae exited the leaf and dropped onto the sand. Also, the location of larval head capsules, which can be seen through the semitransparent upper surface of the leaves, was marked with a pen every 8 h during larval development and the exact time of each mark was recorded. After mature larvae dropped to the soil, mines were dissected and the locations of the head capsules were compared with marks on the leaf surface to determine duration of each instar in each generation and the mine length of each instar was measured.

Mature larvae were immediately collected after dropping to the soil and transferred individually to a modified beverage lid (3-cm-deep, 3-cm-diameter lid, with small holes at the bottom) filled with disinfected, moist, fine sand. Moist cotton gauze was placed over the sand surface to maintain a high humidity. The sand was examined gently every 24 h for the presence of head capsules and pupae. The time from the last larval

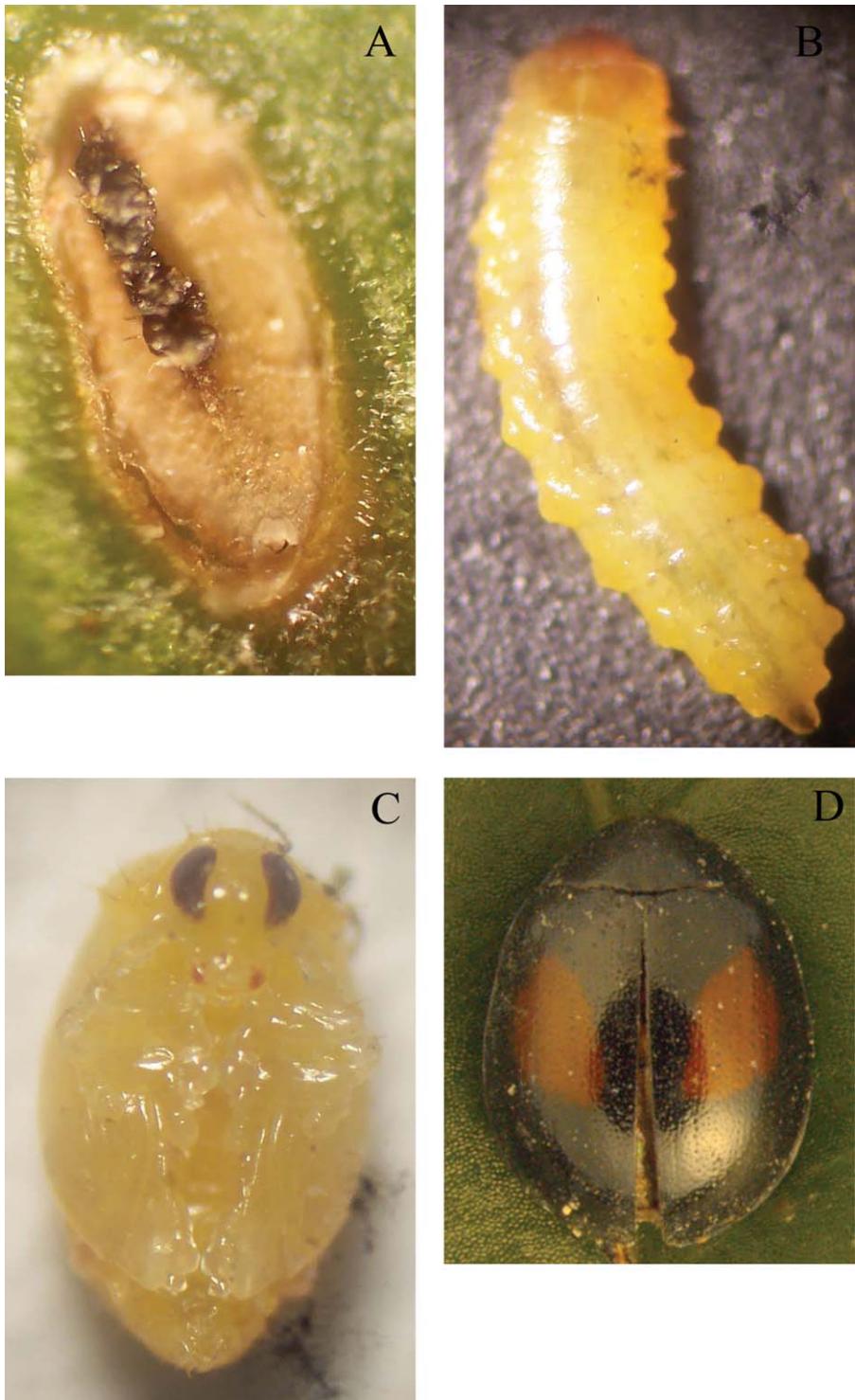


Fig. 1. Photos of life stages and the abdominal sternite of *A. tsekooni* Chen under a stereomicroscope. (A) Egg (80 $\times$ ). (B) Third-instar larva (80 $\times$ ). (C) Pupa (40 $\times$ ). (D) Adult (40 $\times$ ). (E) Abdominal sternites of male (65 $\times$ ), arrow points to the W-shaped apical margin of the terminal sternite. (F) Abdominal sternites of female (65 $\times$ ), arrow points to the evenly rounded terminal abdominal sternite (65 $\times$ ). (Online figure in color.)

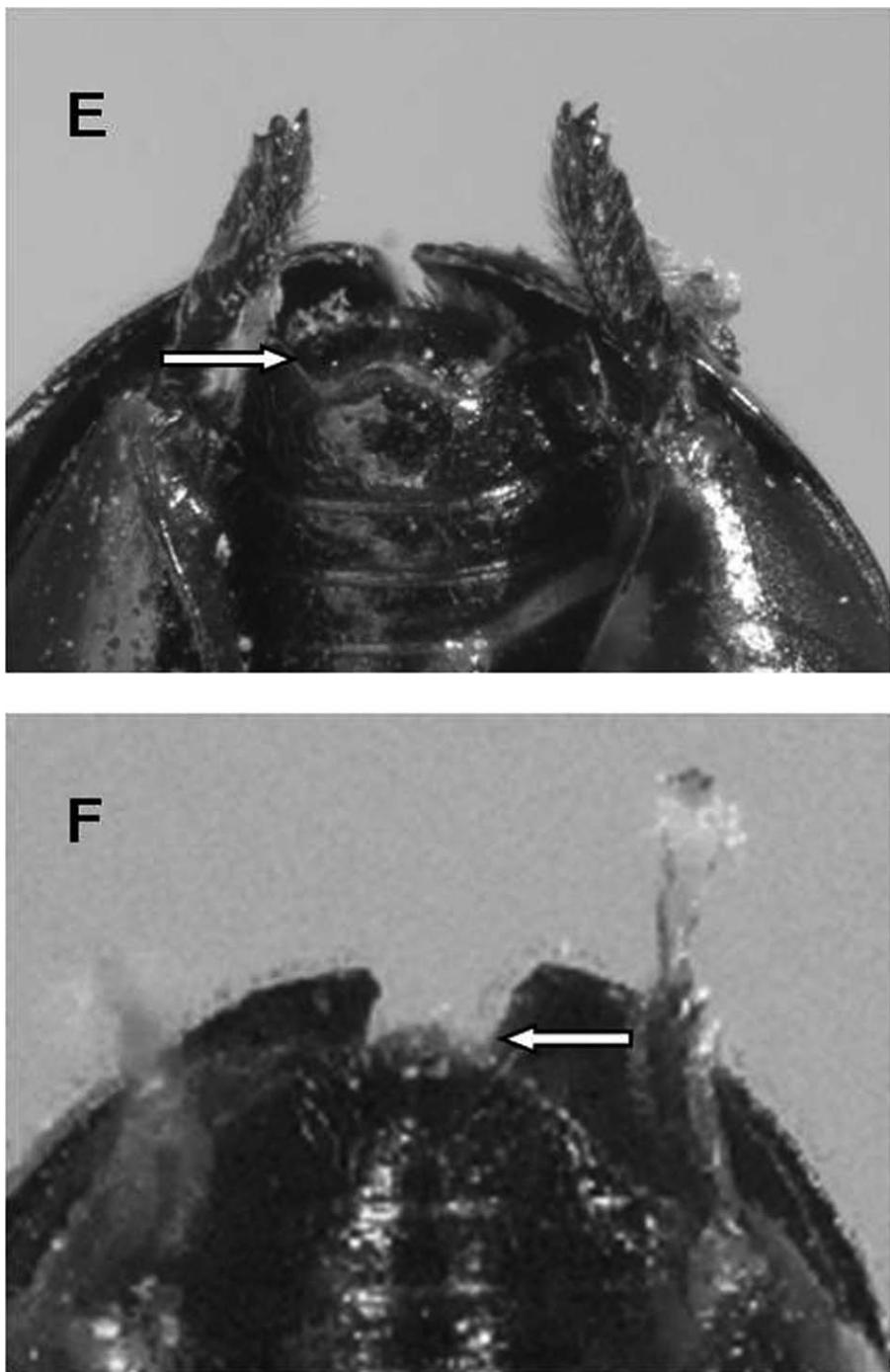


Fig. 1. Continued.

exuviae in the mines to the time of pupation was recorded as the length of the last instar. Duration of pupal development was not determined during this study because the daily disturbance may have adversely affected their development. Instead, another study was conducted to determine the duration of the pupal stage. Modified lids were prepared as described

above, and mature larvae were transferred to the sand and allowed to develop until adults emerged. Pupal duration was the total time minus the duration of the last larval instar.

Larval development of the first generation was measured from late April to late May (average temperature, 21.1°C), the second generation was from late

**Table 1.** Duration of egg, larval, and pupal stages of *A. tsekooni* reared in a shaded, outdoor study area, as well as the average temperature and relative humidity of the study area during various life stages in 2006 in Huangshan City of Anhui Province, China

Stage	Instar	Duration in days, mean $\pm$ SD ( <i>n</i> ) [Temp (°C), RH (%), and time frame during the developmental duration study of each life stages]		
		First generation	Second generation	Third generation
Egg		17.61 $\pm$ 1.88a (50) [17.1°C, 73.5%, 4.10–4.28]	9.37 $\pm$ 1.32b (80) [26.9°C, 78.4%, 6.11–6.21]	7.55 $\pm$ 1.13c (81) [29.4°C, 72.1%, 8.2–8.10]
Larva	First instar	2.86 $\pm$ 0.69a (42)	2.24 $\pm$ 0.42b (65)	1.56 $\pm$ 0.54c (50)
	Second instar	3.70 $\pm$ 0.72a (42)	3.20 $\pm$ 0.44b (65)	2.60 $\pm$ 0.64c (50)
	Third instar (in mine)	5.61 $\pm$ 0.60a (42)	5.3 $\pm$ 0.39b (65)	4.94 $\pm$ 0.91c (50)
	Third instar (in sand, prepupa)	11.21 $\pm$ 0.92a (42) [21.1°C, 75.4%, 4.28–5.22]	10.35 $\pm$ 0.78b (62) [28.5°C, 80.9%, 6.21–7.13]	9.31 $\pm$ 0.91c (32) [29.6°C, 67.9%, 8.10–8.29]
Pupa		12.68 $\pm$ 1.45a (33) [22.7°C, 75.4%, 5.22–6.4]	9.57 $\pm$ 0.74b (53) [29.3°C, 80.6%, 7.13–7.23]	11.58 $\pm$ 1.20c (19) [27.0, 79.7%, 8.29–9.9]
From egg-to-adult emergence		53.67	40.03	37.54

Means within rows sharing the same letters are not significantly different ( $P < 0.05$ ; ANOVA, LSD; SPSS Inc. 2001).

June to mid-July (average temperature, 28.5°C), and the third generation was from mid-August to late August (average temperature, 29.6°C). Pupal development was measured in late May (average temperature, 22.7°C), mid-July (average temperature, 29.3°C), and early-September (average temperature, 27.0°C), respectively. In total, 33, 53, and 19 pupae were used to determine the length of pupal development for the three generations, respectively.

**Adult.** Studies of adult size, leaf consumption, mating behavior and fecundity were conducted in the laboratory (average temperature, 27°C; 70% RH). Adult longevity was observed during routine rearing of *A. tsekooni* in cages with potted plants at the outdoor site. Adult size was determined by measuring the length and width of 73 adults with a calibrated ocular micrometer mounted in a stereomicroscope.

Daily leaf consumption by adults was estimated by placing 10 newly emerged adults in a gauze cage with fresh Chinese privet shoots supplied daily. Leaves fed on by beetles were placed under transparent graph paper with a 1-mm<sup>2</sup> grid to measure the leaf surface eaten. The experiment was replicated 10 times and lasted 1 mo.

Adult fecundity was determined using 42 newly emerged *A. tsekooni* females held individually in gauze sleeves (10 by 20 cm, with the ends sealed by string), which enclosed branches of potted *L. sinense*. Each newly emerged female was paired with three males, and adults were moved to new shoots every 24 h. Dead males were replaced when they were noted. Leaves were examined for eggs with a dissecting scope, and eggs were counted daily until all females died. Mating behavior also was observed during the study.

**Field Observations and Collection.** A field study site was selected near a maize, *Zea mays* L., field in Huangshan City in 2006. Abundant Chinese privets were planted together with other associated plant species such as *Ligustrum lucidum* Ait. and *P. taiwanensis*. At the field site, groups of Chinese privets were planted as brushwood. One sample area included at least eight plants of Chinese privets that were 1.5 m high, with crown diameters of nearly 1.5 m. The field study site was a seminatural location, with no serious distur-

bance history. Beginning in March, Chinese privet in the field was inspected twice per week to determine the appearance of the first overwintered individual. Then, from 20 April to 10 November, systematic field surveys were conducted every 10 d on 20 randomly selected Chinese privet. To minimize disturbance during inspection of the plants, eggs and larvae were counted during a 3-minute time interval without removing them from the leaves. Finally, the crowns of all 20 privet were swept 30 times each with an insect net and all adults collected were counted.

**Data Analysis.** Data for development times of each life stage of *A. tsekooni* in the various generations were subjected to analysis of variance (ANOVA), with means separated by Fisher protected least significant difference (LSD) test (SPSS Inc. 2001).

## Results

**Laboratory and Outdoor Studies.** *Egg.* Eggs of *A. tsekooni* were elongate-ovoid and yellowish when first laid but became dark yellow before eclosion (Fig. 1A). They averaged  $0.62 \pm 0.027$  mm (mean  $\pm$  SD) in length and  $0.28 \pm 0.015$  mm (*n* = 53) in width. Eggs were individually inserted into the leaf tissue on the lower surface, with one to three eggs inserted per leaf. Black wire-shape (long and thin) dejecta could be seen on the surface of the eggs. The egg stage lasted 8–18 d (Table 1), and the duration of egg development was significantly different among the three generations ( $F = 839.412$ ,  $P < 0.0001$ ), with the first generation eggs taking the longest time to develop and the third generation eggs the shortest. The duration of the egg stage for the three generations became shorter as the average temperature increased.

*Larva.* Based on the distribution of head capsule widths (Table 2) of larvae found in the leaf mines and sand, *A. tsekooni* had three larval instars. Larvae completed their first two instars in the mines, whereas the third instar developed partly in the mines and partly in soil. The mine lengths for each instar also are listed in Table 2. The first instar was yellowish white, the second instar was light yellow, and the third instar was golden yellow (Fig. 1B).

**Table 2.** Head capsule width and mine length (mean  $\pm$  SD) of larvae of *A. tsekooni*

Larval stadium	Head capsule width (mm)	Mine length (mm)
First instar	0.212 $\pm$ 0.017 ( <i>n</i> = 109)	8.35 $\pm$ 0.82 ( <i>n</i> = 65)
Second instar	0.370 $\pm$ 0.022 ( <i>n</i> = 109)	15.69 $\pm$ 1.81 ( <i>n</i> = 65)
Third instar	0.490 $\pm$ 0.017 ( <i>n</i> = 111)	53.77 $\pm$ 4.98 ( <i>n</i> = 65)

The larval stage lasted 18–23 d, and the duration of development of each instar differed significantly among the generations ( $F = 65.657$ ,  $P < 0.0001$ ;  $F = 39.786$ ,  $P < 0.0001$ ;  $F = 12.083$ ,  $P < 0.0001$ ;  $F = 44.739$ ,  $P < 0.0001$  for the first-instar larva, second-instar larva, third-instar larva that were in mines, and third-instar larva that were in sand, respectively), with the first generation larvae taking the longest time to develop and the third generation larvae the shortest (Table 1). Each larva produced and occupied only one leaf mine during its developmental time. When the third instars were fully developed, they stopped feeding, cut a hole on the upper surface of the leaf, exited the leaf, and dropped onto the soil. Leaves with long mines often abscised and fell to ground at the same time the mature larva was exiting the mines. After exiting the mines, larvae burrowed into loose soil to a depth of  $\approx 2$  cm and then curled into a C-shaped immobile prepupa for a few days before pupation.

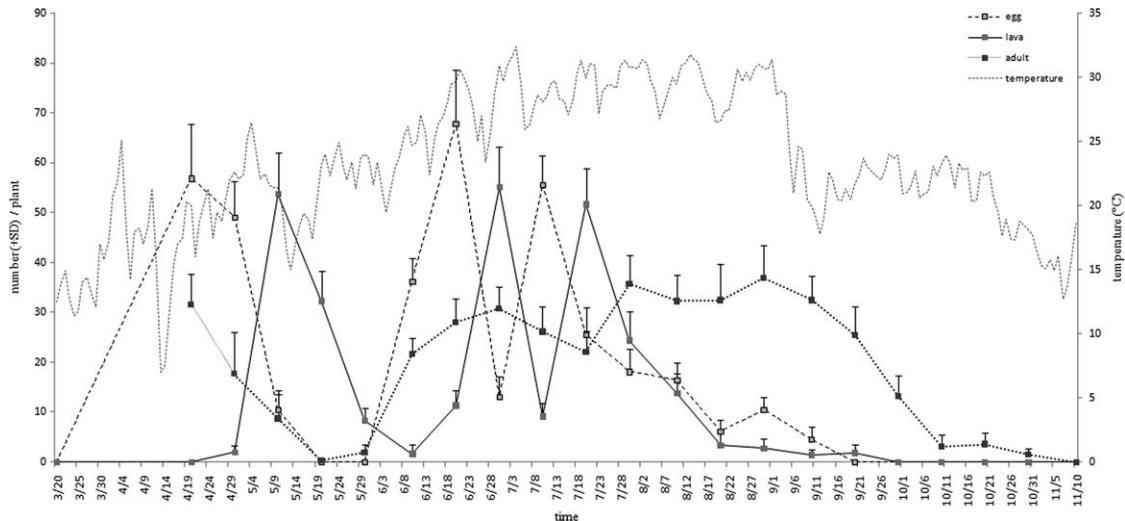
**Pupa.** Pupae of *A. tsekooni* were naked (Fig. 1C), that is, not enclosed in a pupal cell or chamber, and averaged  $2.70 \pm 0.13$  mm in length and  $1.65 \pm 0.10$  mm (*n* = 50) in width. The pupal stage lasted from 10 to 13 d, and this time differed significantly among generations ( $F = 86.838$ ,  $P < 0.0001$ ) (Table 1). The second generation pupae had the shortest pupal developmental time.

**Adult.** Adults averaged  $2.29 \pm 0.20$  mm in length and  $1.48 \pm 0.11$  mm (*n* = 73) in width. Newly eclosed

adults were yellowish brown and gradually darkened over 5 d. They are elongate-oval, convex in lateral view, black with a round red spot in the middle of each elytra, and the head has a distinctly punctuate vertex (Chen 1934) (Fig. 1D). The terminal sternites of males are somewhat W-shaped (Fig. 1E), whereas those of females are evenly rounded (Fig. 1F).

Like larvae, adults fed on the foliage of Chinese privet, but they also fed on the epidermis of twigs when leaves were scarce. Adults fed primarily on the lower surface of leaves forming small holes but seldom penetrated the leaf completely. They consumed an average of  $8.32 \pm 0.96$  mm<sup>2</sup> per d per adult (*n* = 73). Adults underwent a 15-d maturation feeding period before breeding. Copulation occurred mostly at night and lasted  $13.9 \pm 1.6$  h (*n* = 54). Immediately after laying an egg, a female excreted a secretion forming a thin layer directly on the surface of the egg, and then she excreted the black wire-shape dejecta on the eggs. Females laid an average of  $27.8 \pm 5.4$  eggs (*n* = 42) within 30 d. Some adults survived as long as 80 d, but the majority died within 30 d during the growing season. However, overwintering adults live  $>200$  d.

**Seasonal Biology.** Adults overwinter in the leaf litter beneath host plants. During field surveys, hibernation of *A. tsekooni* adults began in September (Fig. 2), although some adults were observed on privet foliage until mid-October despite the low (<20°C) temperatures during that time. The lowest temperature recorded in Huangshan City during our study was 4.9°C in January. Overwintered adults emerged in early April when temperatures were near 15°C and moved onto leaves where they actively fed. Field surveys in 2006 revealed three apparent peaks in numbers of eggs and larvae (Fig. 2). At the initiation of our surveys on 20 April, adults and eggs were already abundant and their numbers decreased sharply to zero 1 mo later. Adult abundance then increased steadily until 30 June



**Fig. 2.** Abundance of various life stages of *A. tsekooni* observed under field conditions in a natural area and the daily average temperature during the experiment in Huangshan City of Anhui Province, China, in 2006.

after which they remained high until 30 August. By the end of September, most adults had either died or entered hibernation (Fig. 2).

### Discussion

Some attempts to control weeds biologically have been successful, whereas others have either failed or produced moderate results. Prediction of biological control success requires biological information about the target plant, the herbivores, and the environment (Julien 1987). Studying and understanding the biology of *A. tsekooni* in its native habitat is therefore important before introducing this agent to the target area for biological control of Chinese privet. Biological studies may also aid in predicting the capacity of this flea beetle to establish and control Chinese privet in a new range.

Eggs of *A. tsekooni* were inserted into leaf tissue, and upon hatching neonate larvae began excavating mines. One to three eggs were laid onto each leaf, which was a low enough density to allow all larvae to complete their development. Similar to other flea beetles, such as *Sphaerodema apicaly* Baly (Zhu 1997) and *Podagricomela weisei* Heikertinger (Zeng 2003) that also mine leaves in their larval stages, eggs of *A. tsekooni* were deposited singly. In contrast, eggs of flea beetles whose larvae are more mobile and do not mine leaves, such as *Haltica lythri* Aubé (Phillips 1977) and *Disonycha argentinensis* Jacoby (Cordo et al. 1984), are deposited in clusters.

We observed females excreting a secretion directly on the eggs forming a layer or film that may prevent desiccation or possibly aid in securing the eggs in the leaves. Like members of the genus *Haltica* Geoff. (Phillips 1977), such as *Haltica lythri* Aubé (Phillips 1977), and other flea beetles, including *Altica fragariae* Nakane (Xue et al. 2007), females deposited a black wire-shape dejecta on each egg. Feytaud (1911) suggested that the dejecta could prevent predation by birds, whereas Phillips (1977) repudiated this, arguing that the eggs were not big enough to be vulnerable to predation by birds. Phillips (1977) argued that the dejecta might contain a deterrent to arthropod predators or parasites. However, Selman (1994) suggested that it may contain chemical cues that could warn other females against ovipositing more eggs, thus increasing the fitness of *A. tsekooni*.

Unlike two closely related species, *Argopistes coccinelliformis* Csiki and *Argopistes biplagiatus* Motschulsky, that excavate several small mines during their larval stages (Inoue 2006), *A. tsekooni* larva develops completely in a single large mine. Occupying a single mine may accelerate leaf abscission, thereby reducing the photosynthetic activity within the leaf that could eventually result in plant mortality. Most flea beetle larvae have three instars, such as *Systema nitentula* Bechyne (Cabrerá et al. 2005), *Disonycha argentinensis* Jacoby (Cordo et al. 1984), and others. The width of larval head capsules is an important parameter for distinguishing various instars, and our results show that *A. tsekooni* also has three instars.

The pupal stage is the most sensitive stage in the life history of *A. tsekooni*. Unlike larvae, pupae were extremely susceptible to injury from handling, which usually caused death. Pupae also were sensitive to changes in humidity. Therefore, determination of optimum environmental conditions for survival of the pupal stage will be essential to successful rearing of this insect for biological control studies.

In our experiments *A. tsekooni* female laid an average of 27.8 eggs over a 30-d period, which was much lower than the closely related species *Argopistes coccinelliformis* Csiki, which laid an average of 235.2 eggs over 189 d (Inoue 1998). It is unclear why *A. tsekooni* fecundity was low in our experiments. Possibly, the experimental conditions were not optimal for *A. tsekooni* females. For example, Inoue (1998) found that daylength was an important factor affecting *A. coccinelliformis* fecundity; thus, the shorter daylength in our laboratory may have affected oviposition of females. However, we suspect that adult survival was shorter under our experimental conditions because of the frequent disturbances resulting from routinely moving females to new branches. In the experiments by Inoue (1998), *A. coccinelliformis* deposited an average of 1.24 eggs per d or 37 eggs over 30 d, which is comparable with that of *A. tsekooni*, which laid an average of 0.93 eggs per d or 28 eggs over 30 d. However, further study of *A. tsekooni* longevity and fecundity are warranted.

*A. tsekooni* had three generations in Huangshan City, Anhui Province, the same number of generations reported in Jiangsu Province in China (Wang et al. 1992). In contrast, *A. tsekooni* was univoltine in the Ibaraki prefecture of central Japan (Inoue 2006), which is located at 36° 09' N, 140° 20' E and has an annual average temperature of 13.3°C. Possibly the lower temperatures prevented *A. tsekooni* from undergoing multiple generations in Ibaraki, Japan, or *A. tsekooni* from Japan and China may be two different biotypes.

According to the climate-matching program CLIMEX (Hearne Scientific Software, Melbourne, Australia), Anhui Province is climatically similar to Atlanta, GA (Sun et al. 2006), which is located at 33° 45' N, 84° 25' W. Therefore, we expect *A. tsekooni* also will undergo multiple generations in the southeastern United States. The multiple overlapping generations of *A. tsekooni* is expected to increase the abundance of this agent and thereby increase the chances of its success as a biological control agent in the new range. Season-long, cumulative defoliation also may aid in the control of Chinese privet in forested settings in which it seems to be most prevalent and grows best under hardwood canopies (J.L.H., unpublished data). Development under a hardwood canopy may allow this evergreen shrub to receive sufficient sunlight during late fall, winter and early spring when the over-story canopy is leafless. Cumulative defoliation that minimizes privet leaf surface area during winter may be important in reducing privet growth and survival.

A few natural enemies of *A. tsekooni* have been reported in China including *Tetrastichus* sp., a larval

parasitoid (Wang et al. 1992). This genus also occurs in the United States, but it is unknown whether U.S. species will parasitize *A. tsekooni*. Likewise, little is known about pathogenic microorganisms associated with *A. tsekooni* in China. Although lack of parasitism would likely increase the chances of success of this flea beetle in the introduced range, some leafminers are severely parasitized by native insects in their new range. For example, two exotic leafminers, *Liriomyza sativae* Blanchard and *L. huidobrensis* Blanchard, introduced into China are parasitized by many indigenous parasitoids (Chen et al. 2006).

Like larvae, adult *A. tsekooni* fed on leaves of Chinese privet. Adult feeding resulted in numerous holes leading to reduced photosynthetic activity and eventually to leaf abscission. At high population densities in late July and August, extensive defoliation was observed, resulting in bare shoots and stems. The number of adults captured by sweep net during this period was almost 40 adults per plant (Fig. 2). Therefore, the combination of larval and adult damage by *A. tsekooni* may provide effective long-term suppression of Chinese privet.

Flea beetles are a large, primarily oligophagous group with several species currently being used as biological control agents of weeds throughout the world. For example, *Aphthona* spp. were released as biocontrol agents for leafy spurge, *Euphorbia esula* (Euphorbiaceae), in North America (Lym and Nelson 2000), and *Agascicles hygrophila* Selman et Vogt was used in China for suppression of *Alternanthera philoxeroides* (Mart.) Griseb., a global virulent weed from South America (Julien et al. 1995). Likewise, *Longitarsus jacobaeae* Waterhouse (Coleoptera: Chrysomelidae) is a flea beetle providing successful biocontrol of the invasive ragwort *Senecio jacobaea* L. (Asteraceae) in northern California and western Oregon (McEvoy et al. 1991, Turner and McEvoy 1995). Adults of *L. jacobaeae* are pit feeders, rasping holes in leaves of mainly juvenile plants, whereas larvae develop by tunneling and feeding in leaves, petioles, stems, and roots (Windig 1991, McEvoy and Coombs 1999).

Although there have been many successes in using flea beetles as biological control agents, a few failures also have been recorded. For example, a promising biological control agent of creeping thistle (*C. arvense*), the flea beetle (*A. carduorum*) from Europe failed to become established in Canada and United States (Peschken et al. 1970, Baker et al. 1972, Schaber et al. 1975). Schaber et al. (1975) concluded that high temperature, low humidity, and predation were the main limiting factors in South Dakota. In Canada, Peschken (1977, 1984) ascribed establishment failure to low temperature and predation. Failures similar to this demonstrate the importance of understanding the biology and habitat requirements of potential biocontrol agents in their native countries.

*A. tsekooni* is a particularly promising biological control agent for Chinese privet and should be tested further for host specificity. Both larval and adult stages are highly damaging to Chinese privet. The beetle also

has multiple overlapping generations per year that should result in significant cumulative defoliation that will aid in suppressing growth and possibly reduce populations of the host plant in its new range. Furthermore, the similarity of climate between Anhui and the southeastern United States suggests that the beetle should be adapted to that region. Preliminary host range tests conducted in China showed that *A. tsekooni* was sufficiently host-specific to be considered for further host specificity testing in the United States (Zhang et al. 2008b).

### Acknowledgments

We are grateful to Prof. Shuyong Wang for assistance with identification of *A. tsekooni* and to Huaijun Xue and Siqin Ge for taxonomic descriptions. We also thank Fang Fang for help with field collection. This research is part of an ongoing Sino-U.S. Chinese privet biological control cooperative program funded by the USDA Forest Service Southern Research Station and partially supported by the National Natural Science Foundation of China (grant 30525009).

### References Cited

- Baker, C.R.B., R. L. Blackman, and M. F. Claridge. 1972. Studies on *Haltica carduorum* Guerin (Coleoptera: Chrysomelidae), an alien beetle released in Britain as a contribution on the biological control of creeping thistle, *Cirsium arvense* (L.) Scop. J. Appl. Ecol. 9: 819–830.
- Burrows, F.J., and J. Kohen. 1986. Inhibition of germination in privet. Plant Prot. Q. 1: 107–108.
- Cabrera, N., A. J. Sosa, J. Dorado, and M. Julien. 2005. *Systena nitentula* (Coleoptera: Chrysomelidae), a flea beetle injurious to *Alternanthera philoxeroides* (Amaranthaceae): redescription, biology, and distribution. Ann. Entomol. Soc. Am. 98: 643–652.
- Chen, S. H. 1934. Revision of the Halticinae of Yunnan and Tonkin. Sinensis 5:316.
- Chen, W. L., H. Peng, Q. Y. Liu, D. Gu, and Z. Z. Li. 2006. Research progress on parasitic natural enemies of leaf-miner in China. Guizhou Agric. Sci. 34: 132–135.
- Cordo, H. A., C. J. Deloach, and M. Ferrer. 1984. Biology and larval host range of the flea beetle *Disonycha argentinensis* (Coleoptera: Chrysomelidae) on alligatorweed in Argentina. Ann. Entomol. Soc. Am. 77: 134–141.
- Dirr, M. A. 1998. Manual of woody landscape plants: their identification, ornamental characteristics, culture, propagation and uses. Stipes, Champaign, IL.
- Feytaud, J. 1911. L'altise de la vigne Biologie-degats-destruction.bulletin de la societe d'études et de vulgarisation de la zoologie agricole. Bordeaux 2: 37–48.
- Georgia Exotic Pest Plant Council. 2003. Top ten exotic pest plants in Georgia. (<http://www.gaeppc.org>).
- Haragan, P. D. 1996. Privet (*Ligustrum vulgare*, *L. sinense*, *L. japonicum*), pp. 58–59. In J. M. Randall and J. Marinelli [eds.], Invasive plants: weeds of the global garden. Brooklyn Botanic Garden, Brooklyn, NY.
- Harrington, T. B., and J. H. Miller. 2005. Effects of application rate, timing, and formulation of glyphosate and triclopyr on control of Chinese privet (*Ligustrum sinense*). Weed Technol. 19: 47–54.
- Inoue, T. 1998. Life history of the flea beetle, *Argopistes coccinelliformis* Csiki (Coleoptera: Chrysomelidae) VI. Overwintering and oviposition abilities of adults which

- emerged late in the season. *Appl. Entomol. Zool.* 33: 21–27.
- Inoue, T. 2006. Seasonal development, oviposition behavior and effects of photoperiod and temperature on oviposition activity in the flea beetle, *Argopistes tsekooni* Chen (Coleoptera: Chrysomelidae). *Jpn. J. Appl. Entomol. Zool.* 50: 33–42.
- Julien, M. H. 1987. Biological control of weeds. A world catalogue of agents and their target weeds. CAB International, Oxon, United Kingdom.
- Julien, M. H., B. Skarratt, and G. F. Maywald. 1995. Potential geographical distribution of alligator weed and its biological control by *Agasicles hygrophila*. *J. Aquat. Plant Manage.* 33: 55–60.
- Lym, R. G., and Nelson, J. A. 2000. Biological control of leafy spurge (*Euphorbia esula*) with *Aphthona* spp. along railroad right-of-ways. *Weed Technol.* 14: 642–646.
- Matlack, G. R. 2002. Exotic plant species in Mississippi, USA: critical issues in management and research. *Nat. Area J.* 22: 241–247.
- McEvoy, P. B., and E. M. Coombs. 1999. Biological control of plant invaders: regional patterns, field experiments, and structured population models. *Ecol. Appl.* 9: 387–401.
- McEvoy, P., C. Cox, and E. Coombs. 1991. Successful biological control of ragwort, *Senecio jacobaea*, by introduced insects in Oregon. *Ecol. Appl.* 1: 430–442.
- Miller, J. H. 2003. Nonnative invasive plants of southern forests: a field guide for identification and control, pp. 20–21. General Technical Report SRS-62, U.S. Dep. Agric. Forest Service, U.S. Department of Agriculture, Forest Service, Southern Research Station, Asheville, NC.
- Peschken, D. P. 1977. Biological control of creeping thistle (*Cirsium arvense*): analysis of the release of *Altica carduorum* (Col.: Chrysomelidae) in Canada. *Biocontrol* 22: 425–428.
- Peschken, D. P. 1984. *Cirsium arvense* (L.) Scop., Canada thistle (Compositae), pp. 139–146. In J. S. Kelleher and M. A. Hulme [eds.], Biological control programmes against insect and weeds in Canada 1969–1980. Commonwealth Agricultural Bureaux, Wallingford, Oxon, United Kingdom.
- Peschken, D. P., H. A. Friesen, N. V. Tonks, and F. L. Bannham. 1970. Release of *Altica carduorum* (Chrysomelidae: Coleoptera) against the weed Canada thistle (*Cirsium arvense*) in Canada. *Can. Entomol.* 102: 264–271.
- Phillips, W. M. 1977. Observations on the biology and ecology of the chrysomelid genus *Haltica* Geoff. in Britain. *Ecol. Entomol.* 2: 205–216.
- Schaber, B. D., E. U. Balsbaugh, Jr., and B. H. Kantack. 1975. Biology of the flea beetle, *Altica carduorum* (Col.: Chrysomelidae) on Canada thistle (*Cirsium arvense*) in South Dakota. *Entomophaga* 20: 325–335.
- Selman, B. J. 1994. Eggs and oviposition in chrysomelid beetles, pp. 69–74. In P. H. Jolivet, M. L. Cox, and E. Petit-pierre [eds.], Novel aspects of the biology of Chrysomelidae. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- SPSS Inc. 2001. SPSS 11 for Windows. SPSS Inc., Chicago, IL.
- Sun, J. H., Z. D. Liu, K. O. Britton, P. Cai, D. Orr, and J. Hough-Goldstein. 2006. Survey of phytophagous insects and foliar pathogens in China for a biocontrol perspective on kudzu, *Pueraria montana* var. *lobata* (Willd.) Maesen and S. Almeida (Fabaceae). *Biol. Control* 36: 22–31.
- Swarbrick, J. T., S. M. Timmins, and K. M. Bullen. 1999. The biology of Australian weeds. 36. *Ligustrum lucidum* Aiton and *Ligustrum sinense* Lour. *Plant Prot. Q.* 14: 122–130.
- Turner, C. E., and P. B. McEvoy. 1995. Tansy ragwort *Senecio jacobaea* (Asteraceae), pp. 264–269. In J. R. Nechools, L. A. Anders, J. W. Beardsley, R. D. Goeden, and C. G. Jackson [eds.], Biological control in the U.S. western region: accomplishments and benefits of Regional Research Project W-84 (1964–1989). University of California, Division of Agriculture and Natural Resources, Berkeley, CA.
- USDA-NRCS [U.S. Department of Agriculture–Natural Resources Conservation Service. 2003. The PLANTS Database. U.S. Dep. Agric.-NRCS, Baton Rouge, LA. (<http://plants.usda.gov/>).
- Wang, Q., X. P. Chu, and D. R. Huang. 1992. The preliminary study of *Argopistes tsekooni* Chen. *Plant Prot.* 18: 25–26.
- Ward, R. W. 2002. Extend and dispersal rates of Chinese privet (*Ligustrum sinense*) invasion on the Upper Oconee River floodplain, North Georgia. *Southeast. Geograph.* 42: 29–48.
- White, D. W., and E. W. Stiles. 1992. Bird dispersal of fruits of species introduced into eastern North America. *Can. J. Bot. Rev. Can. Bot.* 70: 1689–1696.
- Windig, J. J. 1991. Life cycle and abundance of *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae), biocontrol agent of *Senecio jacobaea*. *Biocontrol* 36: 605–618.
- Xue, H. J., S. Y. Wang, W. Z. Li, X. Z. Zhang, and X. K. Yang. 2007. Bionomics of *Altica fragariae*. *Chin. Bull. Entomol.* 44: 69–73.
- Young, J. A., and C. G. Young. 1992. Seeds of woody plants in North America. Dioscorides Press, Portland, OR.
- Yu, J. G., Y. H. Pei, S. M. Wang, and X. G. Sun. 2005. Major species of insect pests, on the garden plant and their damage in Linyi district. *J. Linyi Teach. Coll.* 27: 40–44.
- Yu, P. Y., S. Y. Wang, and X. K. Yang. 1996. Economic insect fauna of China, vol. 54. Coleoptera: Chrysomelidae (2). Science Press, Beijing, China.
- Zeng, Q. 2003. Observation of the biological characteristics and the control technology of *Podagricomela weisei* Heikertinger. *Plant Prot. Technol. Ext.* 23: 26–27.
- Zhang, H. C., and T. W. Yang. 2007. Survey on the diversity of the green plants in the central areas of Huangshan city. *J. Huangshan Univ.* 9: 81–85.
- Zhang, Y. Z., J. L. Hanula, and J. H. Sun. 2008a. Survey for potential insect biological control agents of *Ligustrum sinense* (Scrophulariales: Oleaceae) in China. *Fla. Entomol.* 91: 372–382.
- Zhang, Y. Z., J. L. Hanula, and J. H. Sun. 2008b. Host specificity of *Argopistes tsekooni* (Coleoptera: Chrysomelidae), a potential biological control agent of Chinese privet. *J. Econ. Entomol.* 101: 1146–1151.
- Zhu, Z. H. 1997. Bionomics of *Sphaerodema apicaly* Baly. *J. SW Agric. Univ.* 19: 546–549.

Received 10 July 2008; accepted 7 March 2009.