

Host Specificity of *Argopistes tsekooni* (Coleoptera: Chrysomelidae), a Potential Biological Control Agent of Chinese Privet

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ABSTRACT Chinese privet, *Ligustrum sinense* Lour., is a perennial semi-evergreen shrub that is a serious invasive weed in the United States. Classical biological control offers the best hope for controlling it in an economic, effective, and persistent way. Host specificity of one of the most promising biological control agents of Chinese privet, a flea beetle, *Argopistes tsekooni* Chen (Coleoptera: Chrysomelidae), was evaluated in China by using laboratory no-choice and choice tests on 13 species of Oleaceae and eight species in other families that have important economic value. In adult no-choice survival and oviposition tests, the flea beetle fed and survived for 30 d on *Syringa oblata* Lindl., *Jasminum nudiflorum* Lindl., and three species in the genus *Ligustrum*. Females also oviposited on these species, but only larvae from eggs laid on *S. oblata* and *Ligustrum* spp. developed successfully. In addition, the beetles did not feed or oviposit on the species of economic importance. In choice tests, adults preferred *L. sinense* for feeding and oviposition. These results show that *A. tsekooni* is relatively host specific and warrants further testing as a biocontrol agent of Chinese privet in the United States.

KEY WORDS *Argopistes tsekooni*, flea beetle, weed biological control, host specificity, *Ligustrum sinense*

Chinese privet, *Ligustrum sinense* Lour., is a perennial semideciduous shrub or small tree indigenous to China, Vietnam, and Laos (Wu and Raven 2003, The Nature Conservancy 2004). In its native habitat, it is used as an ornamental, for medicines, teas, and other uses, and it is not considered a pest (OuYang 2003, OuYang and Zhou 2003). Chinese privet was first introduced into the United States in 1852 as an ornamental shrub (Coates 1965, Dirr 1990), but it was recorded as escaping from cultivation in southern Louisiana by the 1930s (Small 1933). During the 1950s, 1960s, and 1970s, Chinese privet became widespread in natural habitats (Wilcox and Beck 2007). Chinese privet has become one of the worst invasive plants in the southeastern United States (Faulkner et al. 1989, Stone 1997) where it is naturalized and considered a severe threat to ecosystems from Texas to Florida, and north as far as the New England states (The Nature Conservancy 2004, University of Connecticut 2004). It is also a pest in Australia and New Zealand (Swarbrick et al. 1999), Argentina (Montaldo 1993), and on several Pacific Islands.

L. sinense is particularly damaging along sensitive riparian areas where it forms a single-species midstory that shades out native understory vegetation (Tennessee Exotic Pest Plant Council 1996), including

many rare species. For example, at least one population of Schweinitz's sunflower (*Helianthus schweinitzii* Torrey and Gray), a federally endangered endemic plant to the piedmont of the Carolinas, has been pushed closer to extinction because of privet's ability to shade out competing plant species (U.S. Fish and Wildlife Service 2008). *L. sinense* also has successfully invaded the limestone cedar (*Juniperus virginianus* L.) glade-woodland complex (Quarterman 1950) of the central basin of Tennessee (Morris 2001). This ecosystem is rich in plant endemism in the southeastern United States (Estill and Cruzan 2001), but it is globally imperiled, in part because of exotic species invasions, including Chinese privet (Noss et al. 1995, Morris et al. 2002). *L. sinense* also can be directly harmful to humans. Respiratory irritation caused by floral odors is common where Chinese privet is abundant (Westbrooks and Preacher 1986).

Mowing and cutting are appropriate for small populations or environmentally sensitive areas where herbicides cannot be used. Repeated mowing and cutting controls the spread of Chinese privet, but it may not eradicate it (Tennessee Exotic Pest Plants Council 1996). Although modern herbicides including glyphosate effectively kill privet (Madden and Swarbrick 1990, Batcher et al. 2000, Harrington and Miller 2005), environmental concerns limit the use of herbicides on public land or in sensitive areas. Environmental concerns over widespread use of herbicides, combined with the vast area infested in the United States, make biological control an attractive option.

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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. 2008). The most promising insect for biological control was the leaf-mining flea beetle *Argopistes tsekooni* Chen (Coleoptera: Chrysomelidae). This flea beetle caused serious damage to Chinese privet when its population was high, and it was the dominant species in the insect community of naturally occurring Chinese privet. Females oviposit into leaves, with only a small part visible at the surface. Newly hatched larvae begin mining between the upper and the lower surface of the leaves, and they continue to eat the tissues as they mature. Adults feed externally by scraping the epidermal layer and the underlying cells, usually penetrating through the leaf and causing a small feeding hole. Holes made by adult beetles and numerous long mines created by larvae often result in leaf abscission (Y.-Z.Z. et al., unpublished data).

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* L. (Euphorbiaceae), in North America (Lym and Nelson 2000); *Altica carduorum* Guer. is a successful biological control agent of *Cirsium arvense* (L.) Scop. (Asteraceae) (Wan et al. 1996); and *Agasicles 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).

A. tsekooni is reported to be strictly associated with glossy privet, *L. lucidum* Ait. and *Syringa oblata* Lindl. var. *giraldii* (Lemoine) Rehd., but more information is not available (Yu et al. 1996). According to the literature, the genus *Argopistes* is exclusively associated with the Oleaceae (Yu et al. 1996).

The purpose of the study is to determine the host specificity of *A. tsekooni* through choice and no-choice feeding and oviposition trials. Establishing host specificity is an important step in evaluating potential biological control agents.

Materials and Methods

Test Plants and Insects. The insects were tested on species closely related to Chinese privet selected according to the now generally accepted centrifugal phylogenetic method proposed by Harris and Zwölfer (1968) and Wapshere (1974) for host specificity testing of biological control agents of invasive plants worldwide (DeLoach et al. 2003). According to the literature on Oleaceae, consensus trees from separate and combined molecular analyses were congruent and agreed well with nonmolecular data (Wallander and Albert 2000), suggesting that the plant species selected for host specificity testing through classical taxonomy were appropriate. Thirteen representative species from seven genera of Oleaceae were selected. Besides the closely related species to *L. sinense*, eight

Table 1. Plant species selected for host specificity experiments with *A. tsekooni*

Family	Species
Cucurbitaceae	<i>Cucumis sativus</i> L.
Ericaceae	<i>Rhododendron simsii</i> Planch.
Fabaceae	<i>Glycine max</i> (L.) Merr.
Malvaceae	<i>Gossypium hirsutum</i> L.
Oleaceae	<i>Chionanthus retusus</i> Lindl. & Paxt. <i>Fontanesia fortunei</i> Carr. <i>Forsythia viridissima</i> Lindl. <i>Jasminum lanceolarium</i> Roxb. <i>Jasminum nudiflorum</i> Lindl. <i>Jasminum sambac</i> (L.) Aiton <i>Ligustrum japonicum</i> Thunb. <i>Ligustrum lucidum</i> Ait. <i>Ligustrum sinense</i> Lour. <i>Osmanthus cooperi</i> Hemsl. <i>Osmanthus fragrans</i> (Thunb.) Lour. <i>Osmanthus marginatus</i> (Champ. ex Benth.) Hemsl. <i>Syringa oblata</i> Lindl.
Poaceae	<i>Zea mays</i> L.
Rubiaceae	<i>Gardenia jasminoides</i> Ellis
Solanaceae	<i>Lycopersicon esculentum</i> L.
Theaceae	<i>Camellia oleifera</i> Abel

important agricultural or ornamental plant species also were selected for testing (Table 1).

Test plants were obtained either from seed, cuttings, transplants of plants growing naturally in the field, nursery stock, or from commercial markets. *Syringa oblata* Lindl. and *Osmanthus fragrans* (Thunb.) Lour. were transplanted into a nearby test field because of their large size. Corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.] and cotton (*Gossypium hirsutum* L.) were grown from seeds. Cucumber (*Cucumis sativus* L.) and tomato (*Lycopersicon esculentum* L.) were obtained as potted plants from a nursery, as were most of the ornamental plants not within Oleaceae (*Rhododendron simsii* Planch., *Gardenia jasminoides* Ellis, and *Camellia oleifera* Abel). *Jasminum sambac* (L.) Aiton (Oleaceae) also were purchased as potted plants. Several other plants (*Fontanesia fortunei* Carr., *Osmanthus marginatus* (Champ. ex Benth.) Hemsl.; *Osmanthus cooperi* Hemsl.; *Forsythia viridissima* Lindl.; *Chionanthus retusus* Lindl. & Paxt.; *Jasminum nudiflorum* Lindl.; *Jasminum lanceolarium* Roxb.; *Ligustrum sinense* Lour.; Japanese privet, *Ligustrum japonicum* Thunb.; and *Ligustrum lucidum* Ait.), were rooted from cuttings in sand under an automatic misting machine in a greenhouse. Once successfully rooted they were transplanted to plastic pots (20 cm in diameter, 15 cm in height). All plants were held under natural day lengths and temperatures in an outdoor site with a shade cloth. 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.

Experiments were conducted in the greenhouse of the Forestry Institute of Huangshan city. The greenhouse was maintained at 25–30°C and 40–60% RH, with a photoperiod of 16:8 (L:D) h. Newly emerged, unfed adults were used to test *A. tsekooni* survival on different plant species. Adults were obtained by digging pupae or newly emerged adults from the soil

under Chinese privet shrubs or trees in a natural area. Pupae or adults were placed individually into clear beakers (12 cm in diameter, 15 cm in height) containing moist fine sand and covered with a screen until the majority of *A. tsekooni* had reached maturity. Adults were sexed by assessing the shape of the last abdominal segment (S. Y. Wang, personal communication).

Adult No-Choice Survival Test. Two mating pairs of adults were selected randomly and placed in 10- by 20-cm polyester organza sleeve bags, which were then securely tied to branches or petioles of leaf clusters of test plants. Two or three sleeve bags were used per plant. The experiment was replicated 10 times for each plant species. Bags with adults were moved to new branches on potted plants every 2–3 d as needed for 30 d, and the area of foliage consumed was measured for each branch. Foliage consumption was quantified by placing transparent graph paper with a 1-mm² grid over each leaf to measure the surface area damaged. Adult survival was recorded after 30 d.

Adult No-Choice Oviposition Test. No-choice oviposition tests were conducted separately from the survival test to eliminate the influence of starvation on adult fecundity. Mature adults, i.e., adults at least 5 d old and feeding normally on Chinese privet, were collected from a natural area and put in a gauze cage filled with fresh branches of Chinese privet for 48 h to ensure they had fed enough for oviposition. Otherwise the methods were the same as the adult no-choice survival test and the flea beetles were allowed to oviposit for 30 d. Eggs deposited in each plant leaf were counted using a dissecting microscope and observed to determine whether eggs hatched successfully.

Adult Choice Feeding and Oviposition Tests. Plant within the Oleaceae family were used for adult choice tests. Fresh branches were inserted through holes in the rubber lid of water filled, 10-ml medical glass vials. These were then placed in 800-ml beakers containing moist cotton or vermiculite and covered with a fine-mesh cloth lid. Each beaker received one *L. sinense* plant branch plus one or two other test plant branches. Two mating pairs of *A. tsekooni* adults were added to each beaker and allowed to feed and oviposit for 1 wk. Each test was replicated 10 times. Foliage consumption and oviposition were qualified as mentioned above.

Data Analysis. Data from adult no-choice and multiple choice feeding and oviposition experiments do not generally conform to the assumptions of parametric tests; thus, we used the nonparametric K-S method of SPSS (SPSS Inc. 2001). Survival, total amount of foliage consumed in 30 d, and number of eggs deposited by each female in adult no-choice tests was analyzed using rank cases and analysis of variance (ANOVA) (SPSS Inc. 2001). Combined, these procedures produced a test that approximated the nonparametric Kruskal-Wallis test (Colpetzer et al. 2004). ANOVA and the least significant difference (LSD) multiple comparison test was used for mean separation in no-choice feeding and oviposition experiments.

Zero data and data produced by beetle testing or exploratory feedings, which were near zero, were excluded (SPSS Inc. 2001). In choice tests, ANOVA and independent-samples *t*-test were used to analyze the amount of foliage consumption and number of eggs deposited after excluding zero data (SPSS Inc. 2001).

Results

Adult No-Choice Survival and Oviposition Tests. In adult no-choice tests, survival of *A. tsekooni* differed significantly among host plants ($F = 185.082$; $df = 21, 198$; $P < 0.0001$). Some adults survived 30 d on *S. oblata*, *J. nudiflorum*, and all three *Ligustrum* spp., whereas no adult survived that long on the other species tested (Table 2). Percentage of survival on *L. japonicum*, *L. lucidum*, and *S. oblata* did not differ significantly from the native host plant *L. sinense*, but survival on *J. nudiflorum* was significantly lower ($F = 20.640$; $df = 4, 45$; $P < 0.0001$).

Foliage consumption by adults also differed significantly in the no-choice test ($F = 66.948$; $df = 21, 198$; $P < 0.0001$). *A. tsekooni* fed normally on *J. nudiflorum*, *S. oblata*, and *Ligustrum* spp., whereas it did not feed on other plants or only fed a small amount to test host suitability. The amount of foliage consumed differed significantly ($F = 889.110$; $df = 4, 45$; $P < 0.0001$) among plants fed upon normally. The ranking of leaf consumption by *A. tsekooni* on the various plants tested was *L. sinense* > *L. japonicum* > *L. lucidum* > *S. oblata* > *J. nudiflorum* (Table 2).

Oviposition also differed significantly in the no-choice test ($F = 204.7$; $df = 21, 198$; $P < 0.0001$). *A. tsekooni* oviposited only on plants within the genus *Ligustrum* and on *S. oblata* and *J. nudiflorum*. Among these plant species, females deposited significantly more eggs on *L. sinense* and *L. lucidum* than any others ($F = 184.716$; $df = 4, 45$; $P < 0.0001$), including *L. japonicum*. Significantly fewer eggs were laid on *S. oblata* compared with the *Ligustrum* spp. Females deposited an average of 0.4 ± 0.69 eggs per female on *J. nudiflorum*, but larval development was unsuccessful. In comparison, all eggs laid on *Ligustrum* spp. and *S. oblata* hatched and larval development was possible, at least to the point where the larvae left the mines and began looking for pupation sites.

Adult Choice Feeding and Oviposition Tests. In choice tests, adult *A. tsekooni* consumed significantly more *L. sinense* than any alternative host except for *L. japonicum* (Table 3). In oviposition tests where *L. sinense* was compared with other *Ligustrum* spp. the beetle demonstrated no preference between *Ligustrum* spp. Females preferred plants within the genus *Ligustrum* for oviposition and deposited no eggs on other test plant species (Table 3; Joseph et al., 1994).

Discussion

Demonstrating host specificity is critical before the introduction of any proposed biological control agents

Table 2. Mean \pm SD percentage of survival, amount of foliage consumed, and number of eggs laid by *A. tsekooni* on various plant species in adult no-choice survival and oviposition tests ($n = 10$)

Plant species	% surviving for 30 d	Foliage consumed (cm ² /beetle/30 d)	Oviposition (eggs/female/30 d)
<i>Cannellia olcifer</i> Abel	0	0 (no feeding)	0
<i>Chionanthus retusus</i> Lindl. & Paxt.	0	0.092 \pm 0.077 (test feeding)	0
<i>Cucumis sativus</i> L.	0	0 (no feeding)	0
<i>Fontanesia fortunei</i> Carr.	0	0.005 \pm 0.007 (test feeding)	0
<i>Forsythia viridissima</i> Lindl.	0	0.002 \pm 0.004 (test feeding)	0
<i>Gardenia jasminoides</i> Ellis	0	0 (no feeding)	0
<i>Glycine max</i> (L.) Merr.	0	0.006 \pm 0.009 (test feeding)	0
<i>Gossypium hirsutum</i> L.	0	0.009 \pm 0.015 (test feeding)	0
<i>Jasminum lanceolarium</i> Roxb.	0	0.083 \pm 0.024 (test feeding)	0
<i>Jasminum nudiflorum</i> Lindl.	22.50 \pm 18.45b	0.320 \pm 0.267e	0.4 \pm 0.699d
<i>Jasminum sambac</i> (L.) Aiton	0	0.010 \pm 0.013 (test feeding)	0
<i>Ligustrum japonicum</i> Thunb.	80.00 \pm 15.81a	6.205 \pm 0.339b	24.20 \pm 4.237b
<i>Ligustrum lucidum</i> Ait.	87.50 \pm 13.17a	4.711 \pm 0.702c	28.30 \pm 3.498a
<i>Ligustrum sinense</i> Lour.	85.00 \pm 17.48a	9.863 \pm 0.409a	30.20 \pm 2.044a
<i>Lycopersicon esculentum</i> L.	0	0 (no feeding)	0
<i>Osmanthus cooperi</i> Hemsl.	0	0 (no feeding)	0
<i>Osmanthus fragrans</i> (Thunb.) Lour.	0	0.027 \pm 0.029 (test feeding)	0
<i>Osmanthus marginatus</i> (Champ. ex Benth.) Hemsl.	0	0 (no feeding)	0
<i>Rhododendron simsii</i> Planch.	0	0 (no feeding)	0
<i>Syringa oblata</i> Lindl.	75.00 \pm 26.35a	0.818 \pm 0.189d	17.9 \pm 2.079c
<i>Zea mays</i> L.	0	0 (no feeding)	0

" Survival and feeding tests were conducted with two newly emerged male and two newly emerged female adults enclosed in sleeve bags with a plant branch. Means within columns sharing the same letters are not significantly different ($P < 0.05$; rank cases and ANOVA, LSD; SPSS Inc. 2001).

Table 3. Mean \pm SD leaf consumption and number of eggs laid on various plant species in adult *A. tsekooni* choice tests ($n = 10$)

Plant species	Consumption area (mm ² /2 pairs adults/wk)	Egg no.
Test 1		
<i>Ligustrum sinense</i> Lour.	176.4 \pm 44.72a	18.5 \pm 5.66
<i>Jasminum nudiflorum</i> Lindl.	1.6 \pm 2.12b	0
<i>Forsythia viridissima</i> Lindl.	0.1 \pm 0.32b	0
Test 2		
<i>Ligustrum sinense</i> Lour.	134.43 \pm 74.82a	22.0 \pm 13.30a
<i>Ligustrum japonicum</i> Thunb.	66.71 \pm 20.29a	16.0 \pm 11.40a
<i>Osmanthus fragrans</i> (Thunb.) Lour.	0	0
Test 3		
<i>Ligustrum sinense</i> Lour.	122.22 \pm 59.00a	28.0 \pm 18.64a
<i>Ligustrum lucidum</i> Ait.	44.11 \pm 29.74b	20.17 \pm 10.11a
<i>Fontanesia fortunei</i> Carr.	0	0
Test 4		
<i>Ligustrum sinense</i> Lour.	103.5 \pm 21.40	16.0 \pm 5.62
<i>Jasminum lanceolarium</i> Roxb.	0	0
<i>Jasminum sambac</i> (L.) Aiton	0	0
Test 5		
<i>Ligustrum sinense</i> Lour.	134 \pm 70.25	15.17 \pm 3.92
<i>Osmanthus cooperi</i> Hemsl.	0	0
<i>Chionanthus retusus</i> Lindl. & Paxt.	0	0
Test 6		
<i>Ligustrum sinense</i> Lour.	144.60 \pm 35.48a	14.00 \pm 2.83
<i>Osmanthus marginatus</i> (Champ. ex Benth.) Hemsl.	0	0
<i>Syringa oblata</i> Lindl.	2.90 \pm 1.73b	0

Means within a test group and column sharing the same letters are not significantly different ($P < 0.05$; ANOVA, LSD).

Each container had two newly emerged male and female *A. tsekooni*, one *L. sinense* plant branch, and branches of two other plant species.

regardless of their control potential (Schroeder 1983). Possible harm to economic and other nontarget plants must be carefully assessed and only insects proven to be host specific are acceptable for release (Balciunas et al. 1994).

Larvae of *A. tsekooni* are leafminers that cannot move to other host plants; thus, it is important to determine the host range and oviposition behavior of the adults, which are also herbivorous. Our tests confirmed that *A. tsekooni* has a narrow diet and host range restricted to the genus *Ligustrum* and *S. oblata*, and possibly other *Syringa* species. Of those plants tested the genus *Syringa* is phylogenetically closest to *Ligustrum* (Wallander and Albert 2000). The preference of *A. tsekooni* supports this and suggests that more distant genera are unlikely to be acceptable as hosts for this insect. *A. tsekooni* did successfully feed and oviposit on *S. oblata* in adult no-choice survival and oviposition tests, but when *L. sinense* was present they fed very little and did not oviposit on *S. oblata*. Lilacs (*Syringa* spp.) are widely planted non-native ornamental species in the United States, so further testing will be necessary to ensure *A. tsekooni* does not cause serious damage to these plants. Like Chinese privet, Japanese privet and glossy privet are considered exotic invasive species in the United States (Miller 2003, Munger 2003); thus, the ability of *A. tsekooni* to feed and oviposit on them should be of less concern. The introduction of *A. tsekooni* also may provide some control of these other invasive exotic weeds.

Recently, ecologists have expressed concerns about the ecological risk of classical biological control (McEvoy 1996; Simberloff and Stiling 1996; Strong 1997; Pemberton 2000; Louda et al. 2003a,b). Some unpredicted side effects have been documented as an un-

desired consequence of a highly limited number of weed biological control programs (Carruthers and D'Antonio 2005). In some cases, biological control agents have potential to reduce biodiversity of native species (Louda et al. 1997). However, where damage to nontarget plant species has occurred, it has resulted from imported insects that adapted to eat physiologically acceptable but less preferred and less suitable host, in situations where the "preferred" host was not present (Louda et al. 2003a, Colpetzer et al. 2004). In our tests, the physiological hosts of *A. tsekooni* were *Ligustrum* spp. and *S. oblata*, which are non-native species in the United States. Generally, most practitioners of biological control believe the ecological host range in the field is narrower than physiological host range (Wapshere 1989, Cullen 1990, Harley and Forno 1992). During observations of *A. tsekooni* in the field, the flea beetles rarely fed on *L. japonicum* adjacent to heavily infested and damaged *L. sinense* (Y.-Z.Z., unpublished data). Thus, *A. tsekooni* seems to be a good candidate for biological control of *L. sinense* in North America and should be evaluated further.

Our results demonstrate the narrow host range of *A. tsekooni*, but we were unable to test North American species; thus, further testing will be necessary. However, this and other studies (Y.-Z.Z. et al., unpublished data) demonstrate the potential of this beetle as a biological control agent for *L. sinense*.

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