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ORIGINAL ARTICLE

Herbivory effects of *Argopistes tsekooni*, a chrysomelid beetle, on container-grown Chinese privet, *Ligustrum sinense*

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Abstract The impact of *Argopistes tsekooni* Chen (Coleoptera: Chrysomelidae), a herbivore, on Chinese privet, *Ligustrum sinense* Lour. (Scrophulariales: Oleaceae), an invasive shrub in the United States, was studied in China. Five densities of adults were inoculated into 3-year-old potted Chinese privet plants in cages under field conditions for 1 month. Plants exposed to high densities of adults were severely damaged and the above-ground portions of some were killed, while the survival rates of adult *A. tsekooni* were comparatively high. The amount of new growth of the main stem, the number of new leaves, and oven-dried biomass were significantly reduced by the combined feeding of larvae and adults. Above-ground plant mortality was 100% when plants were exposed to 24 and 30 adults/plant. In this study *A. tsekooni* had a significant negative impact on Chinese privet growing in pots, which suggests that it may be a promising candidate for biological control of Chinese privet in the field in North America.

Key words biological control, Chrysomelidae, Coleoptera, Oleaceae, plant mortality, pre-release efficacy assessment

Introduction

Chinese privet, *Ligustrum sinense* Lour. (Scrophulariales: Oleaceae), is a semi-evergreen to evergreen shrub or small tree native to China (Miller, 2003). The plant was introduced into North America in 1852 as an ornamental shrub (Dirr, 1998) where it escaped from cultivation and is currently found in 19 states east of the Rocky Mountains, ranging from Massachusetts to Florida and west to Texas (Ward, 2002). Between 1950 and 1980 Chinese privet expanded its distribution at an exponential rate and is now present in over 40% of southeastern US counties (USDA-NRCS, 2003), and is ranked among the top 10 exotic plant pests of Georgia (Georgia Exotic Pest Plant Council, 2003) and Mississippi (Matlack, 2002). The aggressive and troublesome invader often forms dense thickets, particularly in bottomland forests and along fencerows, thus moving into forests, fields and right-of-ways (Miller, 2003). Chinese privet is widely believed to drastically reduce native plant biodiversity because of its ability to shade out native vegetation (USDI Fish and Wildlife Service, 1992; Merriam & Feil, 2002) and form dense, monospecific stands that dominate the forest understory (Dirr, 1998). Large-scale control of privet is labor-intensive and requires the use of large amounts of herbicides (Hanula *et al*., 2009 and references therein). Therefore, biological control may be the most cost-effective and long-term control option.

A cooperative Sino–US Chinese privet biological control program was initiated in 2005. More than

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100 phytophagous insect species were found feeding on Chinese privet (Zhang *et al*., 2008a). One beetle species, *Argopistes tsekooni* Chen (Coleoptera: Chrysomelidae), that appeared to be a promising biocontrol agent is native to China where it is found in Liaoning, Jiangsu, Zhejiang and Hubei Provinces (Yu *et al*., 1996). A preliminary host specificity test showed that it had a relatively narrow host range and warranted further investigation (Zhang *et al*., 2008b).

Female *A. tsekooni* insert eggs into leaves of Chinese privet which hatch in approximately 10 days. Larvae excavate and feed within the leaf mesophyll, forming long, curved mines within leaves. Larvae feed for around 11 days then exit the leaves, drop to the soil and pupate. Adults emerge about 20 days later and feed on the leaves of Chinese privet, forming many small holes that accelerate water loss and wilting. In the field, adults overwinter in leaf litter, emerge in early spring and complete three overlapping generations between April and September (Zhang *et al*., 2009).

Classical weed biological control is a safe, cost-effective and sustainable method of weed control that has resulted in many successes world-wide (McFadyen, 1998). However, ecologists have expressed concerns about the ecological risk of classical biological control, including direct and indirect nontarget effects to ecosystems (McEvoy, 1996; Simberloff & Stiling, 1996; Strong, 1997; Pemberton, 2000; Louda *et al*., 2003a, 2003b). Host-specificity is the crux for determining whether a weed biological control project is likely to succeed without causing more harm than good. Extensive evaluations of host specificity prior to release are now the norm (Blossey *et al*., 2001; Pemberton, 2000), but pre-release impact studies that measure the effects of herbivore feeding on the target invasive plant are rare (McClay & Balciunas, 2005). The lack of pre-release impact studies can lead to introduction of ineffective biocontrol agents, so these assessments are important before introducing a biocontrol agent into the target area. McClay and Balciunas (2005) pointed out that although effects of insect feeding on single plants do not necessarily translate into population-level effects, an agent cannot have a population-level effect without damage at the individual plant level. Therefore, pre-release efficacy assessments are important to understand per-capita effects (Hough-Goldstein *et al*., 2008) and establish the potential efficacy of the biocontrol agent.

To better understand the potential impact of *A. tsekooni* feeding on Chinese privet, experiments were conducted in 2006 using individually potted Chinese privet enclosed in beetle-proof cages with various densities of the flea beetles. We predicted that high densities of beetles would result in greater defoliation of plants that would weaken them.

Materials and methods

The experiment was conducted at a field site in Huangshan City (29°43′N, 118°16′E and elevation 200 m), Anhui Province. The average annual precipitation at the site is 129.5–170.2 cm; the average temperature is 15.4–16.8°C, and the average frost-free period is 230 days (Zhang & Yang, 2007). The climate-matching program Climex (Hearne Scientific Software, Melbourne, Vic., Australia) indicated that this is the province most similar in climate to southeastern US (Sun *et al*., 2006).

The leaf mining habit of larval *A. tsekooni* made it impossible to pick larvae out of the leaf mesophyll without killing them, which prevented separate studies of larval and adult feeding. Therefore, potted Chinese privet was exposed to different numbers of adult *A. tsekooni* for a sufficient period of time to assess the impacts of both adult and larval feeding on plant tissues.

One hundred Chinese privet seedlings of similar age (3 years), height (average height = 39.95 ± 8.78 cm), and vigor (average number of leaves/plant = 200.3 ± 57.68) were selected from a nursery and transplanted individually into 20 cm diameter pots at an outdoor site beneath a shade cloth supported on poles. Seedlings were irrigated daily or as needed and fertilized weekly. Four weeks later, 30 plants similar in height and vigor were selected, divided into six groups at random, and assigned to six different treatments. Treatments consisted of flea beetle densities of 0 (control), 6, 12, 18, 24 and 30 adults (sex ratio 1 : 1) per plant. Each potted plant was covered with a cylindrical cage made of fine polyester organza mesh over an iron wire frame (20 cm diameter and 60 cm high). A twist tie held the top of the cage closed to prevent flea beetles from entering or exiting the cages.

The flea beetle densities for this project were determined by investigating a natural population in the field in June when their population was large and defoliation was high (Zhang *et al*., 2009). To obtain an estimate of *A. tsekooni* field population levels, we enclosed branches of privet with the same type of cylindrical organza cloth cages used to enclose test plants. Enclosed branches were shaken to dislodge adult beetles and the numbers enclosed in the cage were counted. Eighteen adults was the average number collected in this way. Based on this information, we selected densities above and below this level to provide a wide range in which to the study effects of the defoliator.

Adults were collected from Chinese privet in the field and released onto caged plants on July 5, 2006.

Females oviposited on leaves throughout the experiment. We recorded the number of dead plants and surviving insects in each treatment 1 month later when several plants died and the experiment was terminated. Plants were considered dead if the above-ground stems were brittle and incapable of resprouting. We did not determine if the root system and root collar were still living and capable of producing new sprouts.

Total plant height and the number of leaves per plant were measured before and after the experiments. The impact of beetle feeding on plant growth was expressed as the reduction or change in growth of the main stem of the plant (height after beetle feeding minus the height before beetle feeding) and change in the number of leaves (total number of leaves at the end of the experiment minus the total number of leaves before the experiment). Total plant biomass was measured at the end of the experiment by removing the plants from their pots and gently dislodging the soil from the roots using water. The plants were then oven-dried at 70°C for 48 h to obtain their dry mass.

Data were analyzed using the statistical program SPSS (2001) and Microsoft Excel. The data were normal with homogeneous residual variances. A one-way analysis of variance (ANOVA) was used to assess the influence of different adult densities on the change in plant height growth, leaf number and dry mass of Chinese privet. Least significant difference (LSD: $\alpha = 0.05$) was used to separate means if the F-probability from the ANOVA was significant at 5%.

Results

None of the plants treated with the three lowest densities of beetles (0, 6 and 12 adults/plant) died, while above-ground stem mortality was 80%, 100% and 100% for plants exposed to 18, 24 and 30 adults/plant, respectively (Table 1). At the end of the experiment adult *A. tsekooni* survival varied depending upon initial adult densities ($F = 9.013$; $P < 0.001$) (Table 1).

As adult densities increased, plant height growth was reduced dramatically ($F = 12.689$; $P < 0.001$). Feeding by 6, 12, 18, 24 and 30 adults/plant reduced the growth of the main stem by 33%, 72%, 82%, 92% and 100%, respectively, compared to control plants (Fig. 1A).

The change in the number of leaves after 1 month differed significantly among various treatments ($F = 36.76$; $P < 0.001$), decreasing with increased adult density. Plants with 6 and 12 adults/plant had fewer new leaves than controls but only those with 12 adults/plant were significantly lower, while plants with 18, 24 and 30 adults per plant lost more leaves to feeding by *A. tsekooni* than they gained through new growth (Fig. 1B).

The dry mass of plants inoculated with various densities of adults was significantly reduced ($F = 2.556$; $P = 0.054$). Plants inoculated with 6, 12, 18, 24 and 30 adults/plants had reductions in plant dry mass compared to controls of 32%, 44%, 54%, 54% and 54%, respectively (Fig. 1C).

Discussion

Although *A. tsekooni* causes serious damage to ornamental Chinese privet in China, the interaction of the flea beetle and host plant has not been studied in detail. As the process of screening a potential biological control agent for introduction is expensive and many are concerned about potential non-target effects, a global call has arisen to reduce the number of new introductions and to ensure that those agents proposed for release are effective (McEvoy & Coombs, 1999). The quantitative impact of *A. tsekooni* feeding on Chinese privet was studied to enable decisions regarding the prospects and likelihood of success of this beetle in a biological control program targeting Chinese privet in the US.

Both larvae and adults of *A. tsekooni* feed on the leaves of Chinese privet. Because larvae are leaf miners and plants could not be inoculated with them separately, adults of both sexes were inoculated and allowed to oviposit.

Table 1 Number of surviving adults, percent survival and Chinese privet mortality after exposure to various densities of *Argopistes tsekooni* adults for 1 month under natural conditions ($n = 5$).

Variable measured	Initial *A. tsekooni* density (adults/plant)					
	0	6	12	18	24	30
Surviving adult *A. tsekooni* (mean ± SD)	0	5.8 ± 0.45	11 ± 0.71	15.8 ± 0.71	21.4 ± 1.34	22.8 ± 1.30
% Adult *A. tsekooni* survival	–	96.7 a	91.7 ab	87.8 b	89.2 ab	76.0 c
Number of dead plants	0	0	0	4	5	5

Means within rows with the same letters are not significantly different ($P < 0.05$; analysis of variance, least significant difference; SPSS Inc., 2001).

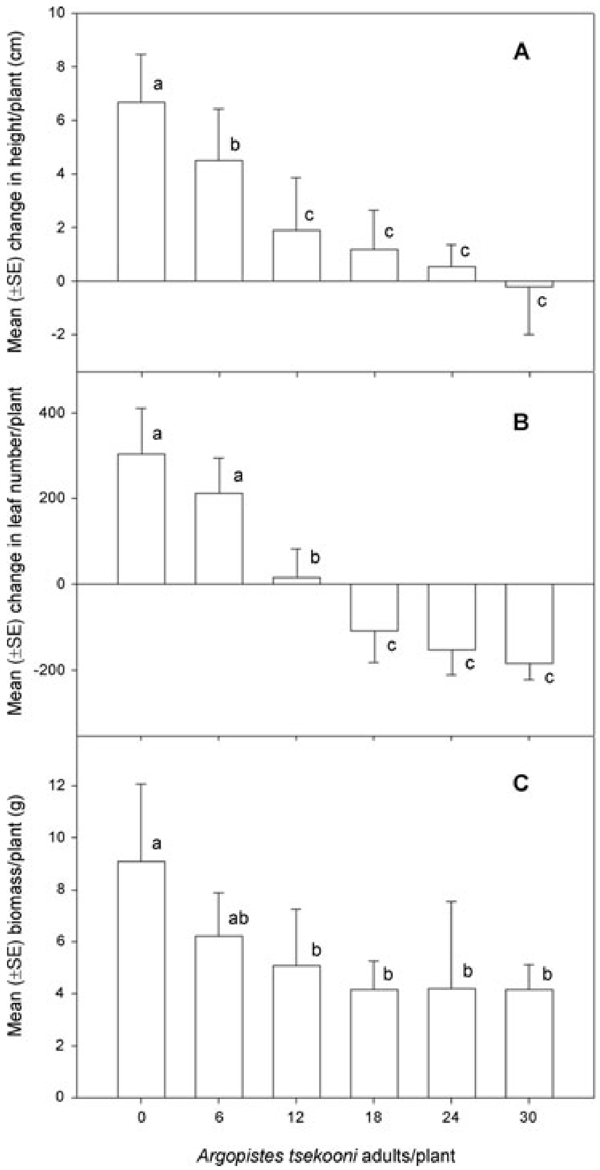

Fig. 1 Impact of 0, 6, 12, 18, 24 and 30 adults/plant on the increase in plant height (A), change in number of leaves (B) and dry mass (C) of Chinese privet plants. Data are means (+ SE) of five replicates per treatment. Bars with the same letter are not significantly different (least significant difference [LSD], $\alpha = 0.05$).

One month was sufficient time to allow for the 10-day egg maturation and 11-day larval feeding period prior to pupation (Zhang *et al*., 2009). Despite the relatively short duration of this experiment the combined feeding of adults and larvae was extensive enough to cause mortality to the above-ground portions of plants grown in containers.

Adult inoculation densities were adequate to observe a range of host plant responses. We followed the procedures of Ding *et al*. (2006) to determine the appropriate densities of adults. They found herbivore densities that were too high resulted in rapid mortality of the host plants, while too conservative herbivore densities did not create the near complete defoliation of plants often observed in the field. Therefore, we chose the adult density of a natural population in the field in June as the middle density for the treatments, allowing us to observe a full range of host responses.

Defoliation caused by leaf-feeding insects greatly reduces photosynthesis and subsequent growth and reproduction of plants. Introducing defoliators to feed on an invasive plant might result in plant control but in some cases plants have been able to compensate or even overcompensate for herbivory, depending on biotic and abiotic conditions and timing and intensity of feeding (Hawkes & Sullivan, 2001; Maschinski & Whitham, 1989; Trumble *et al*., 1993; Wise & Abrahamson, 2005). However, according to van der Meijden (1989) plants have a limited "energy budget" to use to compensate for insect attacks, because other functions such as growth, maintenance and reproduction cannot be stopped completely to allow for the continuous allocation of reserves to compensatory growth. Thus, attacks by leaf-feeding biological control agents, if sufficiently intense and prolonged, should eventually reduce the fitness of plants like Chinese privet.

Results of this experiment showed that, although plants were able to compensate for the damage caused by our low adult density, the combined feeding by larvae and adults of *A. tsekooni* at higher densities reduced the biomass of potted Chinese privet primarily due to defoliation. Adult densities above 18 adults/plant resulted in some mortality and all of the above-ground portions of plants died at densities of 24 and 30 adults/plant. At the end of the experiment, adult beetle survival was relatively high even at the higher inoculation densities, although all surviving adults were concentrated at the tips of shoots whether there were leaves or not (Y. Z. Zhang, pers. obs.). Adult *A. tsekooni* tolerate competition well, based on their relatively high survival in this experiment, although percent survival declined with increasing adult densities, suggesting that starvation was occurring at higher densities.

This experiment was conducted with caged potted plants, which are not likely to accurately represent host plant responses under natural field conditions because artificial confinement of the flea beetle on the plants prevented adult dispersal and possibly predation. On the other hand, the high densities of beetles on single plants resulted in competition that possibly contributed to beetle mortality. Although laboratory experiments give an indication of the effect a biocontrol agent might have on the performance of host plants, they do not give any indication of the effect of an agent on the plant's population dynamics under field conditions (Crawley, 1989). Only careful field monitoring once the agent is released and established can show how well biological control will work (Williams, 2005; Hough-Goldstein *et al*., 2008).

However, our pre-release efficacy assessment of *A. tsekooni* on Chinese privet showed that the candidate agent had a strong impact on the growth and biomass of the target plants, greatly reducing the vigor and fitness of the plants, and even killing the above-ground portions of plants within 1 month when densities were high. We speculate that this flea beetle, once established in climatically favorable areas, could reduce the fitness of Chinese privet through the combined effects of adult and larval defoliation, thus reducing the competitive ability of the plant in natural habitats in the US.

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