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Evaluation of Host Range and Larval Feeding Impact of *Chrysolina aurichalcea asclepiadis* (Villa): Considerations for Biological Control of *Vincetoxicum* in North America

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ABSTRACT A biological control program has been initiated against European swallow-worts *Vincetoxicum nigrum* (L.) Moench. and *V. rossicum* (Kleopow) Barbar., which are invasive in North America. A population of the leaf beetle *Chrysolina aurichalcea asclepiadis* (Villa) originating from the western Alps has been under evaluation as a part of this program. The preliminary host range of *C. a. asclepiadis* was determined among 37 potential host plants. In addition, a prerelease impact study was conducted to determine the effect of larval feeding on the performance of *V. nigrum*. Under no-choice conditions beetle larvae completed development on nine plant species within the genera *Artemisia* and *Tanacetum* (Asteraceae) and *Asclepias* and *Vincetoxicum* (Apocynaceae). The host range of adults is broader than larvae (13 plant species within five genera received sustained feeding). Three of the six nontarget species supporting larval development are native to North America, however in separate oviposition tests, female beetles failed to produce eggs when confined to these hosts. In multiple-choice tests, neither larvae nor adults preferred *Vincetoxicum* spp. to nontarget species. Larval damage by *C. a. asclepiadis* at densities at and above five larvae per plant substantially reduced growth, biomass, and delayed reproduction of *V. nigrum*. However, this population of *C. a. asclepiadis* is polyphagous and unsuitable for biological control of *Vincetoxicum* because of potential risk of attack to *Asclepias tuberosa* L. and native North American Asteraceae, particularly *Artemisia*.

KEY WORDS host specificity, prerelease impact, nontarget effects, Chrysomelidae, swallow-worts

Over the past 30 yr the distribution of two European swallow-worts (genus *Vincetoxicum*, family Apocynaceae) has expanded dramatically in North America. Swallow-worts were introduced into North America from Europe as ornamentals during the mid-19th century (Moore 1959, Pringle 1973, Sheeley and Raynal 1996) and are currently distributed throughout central and northeastern United States and in the Canadian provinces of Ontario and Quebec (DiTommaso et al. 2005). Black swallow-wort, *V. nigrum* (L.) Moench. (synonym: *Cynanchum louiseae* L.), is native to the Mediterranean region, whereas pale swallow-wort, *V. rossicum* (Kleopow) Barbar. [synonym: *C. rossicum* (Klepow) Borhidi] is native to Ukraine and southwestern European Russia (Pobedimova 1952, Markgraf 1972).

Swallow-worts are long-lived, polycarpic plants that can self-pollinate. Their high reproductive potential coupled with a strong ability to compete for resources has enabled them to spread to a variety of habitat types in North America. Both species readily invade disturbed habitats and are commonly observed in grasslands, pastures, stream banks, cliffs, and encroaching on agricultural row crops (DiTommaso et al. 2005). Swallow-worts alter habitats by smothering native vegetation (Lawlor 2000, Cappuccino 2004, DiTommaso et al. 2005, Douglass et al. 2009) and dense populations threaten sensitive plant communities of endemic flora (DiTommaso et al. 2005). Moreover, swallow-wort infestations are inhabited by a depauperate array of invertebrates that may affect local food webs in comparison to unaffected areas (DiTommaso et al. 2005, Ernst and Cappuccino 2005). Swallow-worts are commonly regarded as weeds of natural areas; however, unabated population expansion also threatens productivity of pastures and no-till agricultural systems (DiTommaso et al. 2005).

Swallow-wort control is difficult because of a large, persistent rootstock and rapid response to disturbance. Mowing plants during flowering will reduce seedpod production (McKague and Cappuccino 2005), but control is temporary and not selective. Annual herbicide treatment only produces temporary results (Lawlor and Raynal 2002, Averill et al. 2008) and application costs and risk to nontarget species has promoted interest in alternative control techniques. A classical biological control program was initiated for swallow-worts in North America in 2006 (Tewksbury et al. 2002, Weed et al. 2011b). During surveys for potential biological control agents of *Vincetoxicum* in

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Europe, the leaf beetle *Chrysolina aurichalcea asclepiadis* (Villa) was observed attacking *V. hirundinaria* in southern Switzerland (Weed et al. 2011b). It was later discovered that this beetle successfully develops on *V. nigrum* and *V. rossicum*, prompting further evaluation as a biological control agent (Weed et al. 2011b).

Chrysolina a. asclepiadis is a univoltine leaf beetle distributed throughout the Western Alps, Lombardy, and the Adriatic coast where it attacks *V. hirundinaria* (Bieńkowski 1998, 2001). The life cycle of *C. a. asclepiadis* is similar to *C. aurichalcea aurichalcea* (Gebler), which feeds on *Artemisia princeps* Pampan in Japan (Hayashi et al. 1994, Bieńkowski 1998). The population under study has a fall breeding behavior similar to *A. princeps*, which synchronizes egg hatch with shoot emergence in the spring (Hayashi et al. 1994). In early spring, *C. a. asclepiadis* larvae hatch and feed in loose groups on newly expanding leaves of *V. hirundinaria* (Weed 2010b). Pupation occurs in the soil, after which adults emerge for a brief period in early summer to feed on leaves and then return to the soil during reproductive diapause. Adults re-emerge in autumn and mate and lay eggs in clusters on the ground or in leaf material near the host plants (Bieńkowski 1998). However, subtle differences in life history may occur across the geographical range of *C. a. asclepiadis* similar to other *Chrysolina* spp. (Rizza and Pecora 1984, Campbell and McCaffrey 1991, Coombs et al. 2004).

Chrysolina a. asclepiadis is placed in the taxonomically challenging *C. aurichalcea* species complex (Bieńkowski 1998, 2001), which consists of multiple cryptic species with various host plants affiliations across the Palearctic region (Bieńkowski 1998). Some subspecies of *C. aurichalcea* prefer Asteraceae (Hayashi et al. 1994, Bieńkowski 1998) and others attack different host plants depending on geographic location. For example, *C. a. bohemica* Mannerheim attacks *V. hirundinaria* in central Europe but prefers *Artemisia absinthium* L. in the eastern Alps (Kippenberg and Döberl 1994, Bieńkowski 1998). Apparently geographical barriers are largely responsible for local host plant adaptation of *C. aurichaclea* (Bieńkowski 1998) because adults rarely fly (Suzuki 1978) and long distance dispersal is unknown.

Little is known concerning the host specificity of *C. a. asclepiadis.* Specimens from Ticino, Switzerland have only been observed attacking *V. hirundinaria* (Weed et al. 2011b). Similar to other *C. aurichalcea*, movement and host selection behavior of *C. a. asclepiadis* are not well understood. Larvae are attracted to short distance cues associated with host plant volatiles of *Vincetoxicum* and conspecific feeding (Weed 2010b). With the reported variation in host use among beetle subspecies (Bieńkowski 1998), it was important to evaluate the host specificity of *C. a. asclepiadis* to predict potential nontarget effects to native North American plants.

In this study the preliminary host range of *C. a. asclepiadis* was assessed to determine if species outside of the genus *Vincetoxicum* are at risk of attack in North America. We tested the most likely alternate host plants and conducted a prerelease impact study to evaluate plant response to herbivory and provide an indication of whether the herbivore has a negative effect on plant demography (McClay and Balciunas 2005).

Materials and Methods

Insect Culture. Adults of *C. a. asclepiadis* (determined by Andrezej Bieńkowski, Moscow, Russia) from Ticino, Switzerland (46° 26.016′ N, 08° 51.537′ E) were collected in 2006 to establish a research colony in the insect quarantine facility at the University of Rhode Island. After the culture initially was established in 2006 the following procedures also were used from 2007 to 2009 to maintain the culture: Eggs obtained from adults were overwintered at 4°C in petri dishes from November to April of each year. Larvae and adults were reared on *V. hirundinaria*, *V. nigrum*, and *V. rossicum* (plant culture maintenance described below). Because larvae establish better in small groups (Weed 2010b) neonates were transferred in groups of five or ten to freshly cut sprouting shoots held in 473-ml clear plastic jars fitted with water pics. Containers were closed with ventilated clear lids and held under a photoperiod of 16:8 (L:D) h and at ambient room conditions. Larvae were checked daily at which time dishes were cleaned and plant shoots replenished. During the L4 stage, 2 cm of moist, sterilized vermiculite was added into the containers as a pupation substrate. Emerging adults were sexed and maintained on cut shoots of *V. hirundinaria*, *V. nigrum*, and *V. rossicum* fixed into moist florist foam that was held within clear, plastic cylinders (1.3 liters) and provided 2-cm-wide paper strips as an oviposition substrate. Eggs were collected every week from cylinders with a fine-tip brush and transferred to 10-cm petri dishes lined with moistened filter paper.

Test Plant List. A test plant list of 57 species comprised of 36 native North American species and 21 introduced taxa of economic importance has been approved by the Technical Advisory Group (TAG) to USDA-APHIS for host specificity testing of *Vincetoxicum* biological control agents (Milbrath and Biazzo 2007). We elected to initially screen *C. a. asclepiadis* against a subset of key species from the TAG list to determine whether further host range testing was warranted. Our test list (Table 1) also included several species not on the TAG list. Five species within the genus *Artemisia* (Asteraceae) also were included because closely related beetle species are known to attack members of this plant genus (Hayashi et al. 1994). Common tansy, *Tanacetum vulgare* L. (Asteraceae), was also included as an additional taxon of Asteraceae. Thus, our test plant list included 37 species distributed within 23 genera and four plant families of which, the Apocynaceae was represented by 29 species distributed within four subfamilies, eight tribes, and six subtribes. The list also included seven taxa considered either of special concern, threatened, or endangered.

Test plants primarily were grown from seed but some field-collected rootstock and vegetative cuttings

Table 1. Host range of *Chrysolina a. asclepiadis* evaluated in no-choice trials

Plant species	Larval development to pupation			Adult feeding	Oviposition[e]
	n[a]	(% ± SE)[b]	(d ± SE)[c]	(% leaf area eaten ± SE)[d]	
Family Apocynaceae					
Subfamily Asclepiadoideae					
Tribe Asclepiadeae					
Target weeds (Subtribe Tylophorinae)					
Vincetoxicum nigrum (L.) Moench.	6	76.7 ± 6.3	22.5 ± 0.7	27.5 ± 6.3abc	+(7)
Vincetoxicum rossicum (Kleopow) Barb.	6	88.7 ± 4.2	22.8 ± 0.4	31.9 ± 5.4ab	+(6)
Other Tylophorinae					
Vincetoxicum hirundinaria Medik.	6	75.4 ± 7.1	21.6 ± 0.8	34.4 ± 4.7a	+(5)
Subtribe Asclepiadinae					
Asclepias curassavica L.	2	0		5.0 ± 1.5c	NT
Asclepias fascicularis Dcne.	7	0[f]		3.1 ± 1.2	NT
Asclepias fruticosa L.	6	0		1.9 ± 0.8	NT
Asclepias incarnata L	7	0		9.6 ± 3.9abc	NT
Asclepias speciosa Torr.	2	0		21.3 ± 9.8abc	NT
Asclepias syriaca L.	2	0		2.8 ± 1.1	NT
Asclepias tuberosa L.	8	15.0 ± 4.6	29.5 ± 1.6	39.3 ± 8.0a	−(2)
Asclepias viridiflora Raf.	3	0		0	NT
Subtribe Cynanchinae					
Cynanchum laeve (Michx.) Pers.	7	0		0.6 ± 0.4	NT
Subtribe Gonolobinae					
Matelea suberosa (L.)	5	0		4.4 ± 1.2	NT
Subtribe Metastelmatinae					
Funastrum angustifolium (Pers.) Liede & Meve	3	0		3.8 ± 1.2	NT
Subtribe Oxypetalinae					
Araujia sericifera Brot.	5	0		0	NT
Tribe Ceropegieae					
Ceropegia woodii Schltr.	2	0		0.3 ± 0.3	NT
Stapelia gigantea N.E. Br.	2	0		0	NT
Tribe Marsdenieae					
Hoya carnosa (L. f.) R. Br.	4	0		1.9 ± 0.4	NT
Subfamily Periplocoideae					
Periploca graeca L.	5	0		0	NT
Subfamily Apocynoideae					
Tribe Wrightieae					
Nerium oleander L.	4	0		6.4 ± 2.4c	NT
Tribe Malouetieae					
Pachypodium lamerei Drake	3	0		2.5 ± 1.3	NT
Tribe Apocyneae					
Apocynum cannabinum L.	4	0		0.9 ± 0.5	NT
Trachelospermum jasminoides (Lindl.) Lem.	4	0		3.1 ± 2.1	NT
Subfamily Rauvolfioideae					
Tribe Vinceae					
Amsonia tabernaemontana Walter	8	0		0.9 ± 0.5	NT
Vinca minor L.	4	0		0	NT
Tribe Plumerieae					
Allamanda cathartica L.	4	0		0.5 ± 0.2	NT
Plumeria rubra L.	2	0		0	NT
Tribe Carisseae					
Carissa macrocarpa (Eckl.) A. DC.	3	0		0.5 ± 0.2	NT
Carissa grandiflora A.DC.	3	0		0	NT
Family Asteraceae					
Artemisia absinthium L.	3	60.0 ± 29.3	25.4 ± 1.0	14.4 ± 3.6abc	+(7)
Artemisia caudata Michx.	5	8.0 ± 4.9	38.0 ± 1.0	13.6 ± 0.7abs	−(3)
Artemisia ludoviciana Nutt.	5	24.0 ± 10.3	25.3 ± 2.0	8.8 ± 1.6bc	−(5)
Artemisia stelleriana Besser	6	0		4.1 ± 1.0	NT
Artemisia vulgaris L.	7	34.0 ± 11.2	20.0 ± 1.0	19.4 ± 2.6abc	+(5)
Tanacetum vulgare L.	8	56.7 ± 5.4	17.8 ± 0.8	21.9 ± 3.4abc	+(4)
Family Gelsemiaceae					
Gelsemium sempervirens (L.) St. Hil.	4	0		1.3 ± 0.5	NT
Family Loganiaceae					
Spigelia marilandica (L.) L.	3	0		1.6 ± 0.5	NT
Total no. plant species tested		37		37	9

[a] Number of replicates tested for larval feeding per plant species (five larvae per replicate).
[b] Larval survival among replicates on each plant species.
[c] Larval developmental period (d) among replicates on each plant species.
[d] Mean defoliation by eight adults (4♂ and 4♀). Different letters indicate significant differences at $\alpha = 0.05$ among species with >5% leaf area consumed (Dunn's multiple comparison of ranks test).
[e] Presence (+) or absence (-) of oviposition on host plant species with no. of females tested in parentheses. NT = not tested.
[f] One larva developed to L2 stage.

were used to initiate cultures, depending on the species. Seeds were sown in the greenhouse and transplanted after reaching four to six leaf nodes. Rootstocks immediately were transplanted and cuttings were held in mist tents and transplanted when rooted. All plants were transplanted into 1.9- or 2.8-liter pots containing Metro-mix 510 (Sun Gro Horticulture, Bellevue, WA) and kept in greenhouses or in outdoor raised beds located at the University of Rhode Island in Kingston before testing. Plants were watered as necessary and treated with liquid fertilizer (Peter's Professional All Purpose Plant Food, 20N-20P-20K, Madison, WI) every 2 wk during the growing season. Hardy plants were maintained outdoors over the winter in sawdust-filled raised beds covered with an insulating fabric that was removed in spring. Plants unable to survive winter temperatures were kept in the greenhouse until spring.

Host Specificity Testing

No-choice Larval Development. Larvae of *C. a. asclepiadis* were exposed to leaf material of 37 test plant species to determine the range of plants suitable for larval development. Each replicate consisted of five larvae confined to excised leaves of each test plant with petioles immersed in waterpics and held in clear, ventilated 473-ml cups. Larvae were provided young foliage clipped from the top three leaf nodes of test plants because they attack newly expanding leaves in the field. Larvae used for host range testing were taken from bulk egg collections from the rearing colony. Approximately 2 wk before testing, overwintering eggs were moved from incubators to ambient laboratory conditions to induce hatching. Larvae either were transferred immediately to test plants after hatching or held without plant material at 12°C for no longer than 48 h. After transfer, cups were checked daily for survival and provided new foliage as necessary until they completed development. Head capsule size of dead larvae was used to determine the extent of development before mortality. During the L4 stage, 2 cm of moist, sterilized vermiculite was added into the containers as a pupation substrate. Then cups were searched daily to determine the developmental times of larvae. Plants (2–8 replicates per species) were screened during the spring of 2007 and 2008. The number of plant species tested each year varied depending on plant availability. We attempted to screen all replicates of a plant species during the same year but this was not always possible. *Vincetoxicum nigrum*, *V. rossicum*, or *V. hirundinaria* were used as controls because larval survival is similar among these plant species (Weed et al. 2011b). The suitability of host plants for development was evaluated by counting the number of larvae surviving to pupation and calculating the average time to pupation for all individuals per cup. Larval survival (%) and developmental period (d) to pupation are expressed as averages across all replicates for each plant species. Survival and developmental period were not compared among host plants because testing occurred over 2 yr.

No-choice Adult Feeding and Oviposition. Adult feeding preference was evaluated among all plant species under no-choice conditions (Table 1). Adults used in this experiment were obtained from the research colony and reared as larvae on *V. nigrum* and *V. rossicum.* Newly emerged adults were separated by sex into 1.3-liter cylinders and provided fresh *V. nigrum* leaves in an incubator set at 12°C (a photoperiod of 14:10 [L:D] h) for 2 d and then starved 48 h before the experiment. One adult was placed into a 10-cm petri dish lined with moistened filter paper to which a host leaf then was added. The feeding behavior of eight beetles (four males and four females) was assessed on each plant species for 48 h. After 48 h, the percentage of leaf area consumed visually was estimated to the closest 5%. When <5% of the total leaf area was consumed, this damage was considered exploratory feeding; anything more was considered sustained feeding. The percentage of leaf area consumed was compared among those host species with sustained feeding using a Kruskal–Wallis test (JMP 8.0.2, SAS Institute 2009).

Tests were conducted to determine whether the plant species supporting complete larval development in the no-choice tests also were suitable hosts for oviposition. Newly emerged adults obtained from the no-choice larval development trials were immediately sexed and monitored for oviposition using the same setup as in normal insect rearing. The presence of eggs was monitored on individual mating pairs that were provided with fresh leaf material of the larval host plant species. Because males were never obtained from some test plant species during the larval development tests, we added males raised on *Vincetoxicum* into female cages when needed. The number of females monitored for oviposition varied per test plant species (two to seven females per plant species) because of variable larval survival in the no-choice trials. Monitoring of egg production stopped when the female died or 2 wk after the first eggs were found.

Multiple-choice Larval and Adult Feeding. The feeding preference of newly emerged larvae and starved adults was tested among eight plant species supporting larval development in the no-choice tests. We excluded the ninth larval host *Art. caudata* Michx. from the test because its leaves are dissected into many long, slender leaflets, which made it difficult to expose beetles to a suitable leaf area within the confined space. Feeding preference was tested using pieces of test plant leaves held in 10-cm petri dishes lined with moistened filter paper. We exposed insects to either 2-cm leaf discs or an approximately equivalent amount of leaf area of the species with irregular shaped leaves. Before testing, all leaves were scanned onto a computer and leaf area before feeding was calculated using ImageJ software (NIH, Bethesda, MD). After scanning, one leaf disc of each test species was randomly located along the margin of the petri dish. An analysis of variance (ANOVA) was conducted to assess whether a similar leaf area of each plant species was exposed to insects (PROC GLM, SAS Institute 2008).

Twenty arenas were setup each for beetle larvae and adults. In the larval preference test, five neonates were released into the center of 10 arenas and allowed to feed for 72 h. In the adult choice test, a single adult was transferred into 10 arenas (five with females and five with males) and allowed to feed for 48 h. The remaining arenas were designated as controls to correct for autogenic losses in leaf area (Manly 1993, Prince et al. 2004). All leaf discs were rescanned after the feeding period to determine leaf area.

The amount of leaf area consumed in the experiments was determined by correcting for autogenic loss. Feeding preference among plant species of each life stage was analyzed using the procedure of Lockwood (1998). First, the total leaf area consumed of all plant species within an arena was calculated. Then, leaf area consumed of each plant species was divided by this sum to determine the relative proportion of feeding damage to each plant. This proportion was compared among plant species using Hotelling's T^2 test and 95% confidence intervals adjusted for multiple comparisons were calculated around mean differences (Lockwood 1998). Initially, the analysis of adult preference was conducted separately for each gender. However, feeding preferences were similar between genders so the final analysis was conducted with pooled data in Microsoft Excel 14.02 (2010 Microsoft Corp.).

Impact of Larval Feeding on Performance of *V. nigrum*. The effect of larval feeding by *C. a. asclepiadis* on the performance of *V. nigrum* was tested in quarantine. While plants were still dormant in late March 2009, ≈50 rootstocks of *V. nigrum* were collected from Charlestown, RI, and immediately transplanted into 1.1-liter pots with Metro-mix 550 soil (Sun Gro Horticulture, Bellevue, WA). Transplanted rootstocks were grown in the greenhouse for 4 wk and then brought into quarantine. Forty-five plants were randomly allocated to five larval density treatments: 0, 5, 10, 20, and 25 larvae per plant with nine replicates per larval density. Before infestation, the number of shoots, maximum shoot height, and shoot base diameter was measured from each plant. One-way ANOVA confirmed that maximum shoot height (10.1 ± 0.7 cm, mean ± SE) ($F = 0.64$; df = 4, 44; $P = 0.637$), the number of shoots (2.0 ± 0.1) ($F = 1.12$; df = 4,44; $P = 0.335$), and shoot base diameter (2.9 ± 0.1 mm) ($F = 1.66$; df = 4, 44; $P = 0.177$) of plants was similar among treatments.

Before infestation a clear, plastic tube (16 by 11 cm in diameter) was carefully placed around the plant shoots and dug into the soil to confine larvae to plants. Tubes were secured to pots by using Parafilm (Pechiney Plastic Packaging Company, Chicago, IL). In early May the appropriate number (0, 5, 10, 20, 25) of newly emerged larvae was transferred to plants using a fine-tip brush. Each tube was closed with a screened lid. Plants were randomly arranged on a shelf held under a photoperiod of 14:10 (L:D) h at ambient laboratory conditions (16–28°C and 15–50% RH). Plant shoots were kept within 10 cm of fluorescent lights (Daylight model no. F48T12DH0, General Electric, Fairfield, CT) at all times by lowering the shelf during the course of the experiment to accommodate for plant growth. Plants were watered twice per week.

Every 7 d the height of all shoots and the location of larval damage (apical or basal) was recorded. Presence of reproductive structures was checked daily to determine the number of days to flowering and fruiting. Twenty-eight days after infestation, shoot height, the number of main shoots, and the number of reproductive structures were recorded from all plants. We also counted and measured the length of all shoots growing from the axillary buds at the base of the leaf petioles on each plant as a measure of compensation to herbivory. The percentage of leaf area removed was visually estimated to the nearest 10% by two observers and compared among treatments by using a Kruskal–Wallis test. Aboveground plant parts were clipped and dried at 50°C until constant weight and then reweighed.

The effect of larval density on maximum weekly shoot height was analyzed with repeated measures ANOVA. Aboveground biomass and the relative growth rate of stems over the experiment ([final height − initial height]/initial height) were compared among larval densities by using one-way ANOVA (PROC MIXED, SAS Institute 2003). Differences in the number and length (total cm per plant) of axillary shoots, proportion of plants flowering, and number of flowers per plant were compared among larval densities using a Kruskal–Wallis test followed by Dunn's multiple comparison of ranks test (Dunn 1964). Data were transformed as necessary to meet assumptions of the analyses (normality and homoscedasticity), but back-transformed values are presented in tables and figures.

Results

Host Specificity Testing

No-choice Larval Development. Nine plant species distributed within four genera and two families supported complete larval development (Table 1). Of these species, *Art. caudata*, *Art. ludoviciana* Nutt., and *As. tuberosa* L. are native to North America with the latter being the only suitable larval host within the Apocynaceae in addition to the target weeds (Table 1). One larva of 35 survived to the L2 stage on *As. fascicularis* Dcne. but died shortly after molting. Larval survival was highest on *Vincetoxicum* followed by *Art. absinthium* and *T. vulgare* (Table 1). However, average larval developmental period tended to be shortest for *T. vulgare* followed by *Art. vulgaris* L. and *Vincetoxicum* spp. Beetles took ≈9 d longer to finish development on North American versus Eurasian hosts.

No-choice Adult Feeding and Oviposition. Thirty plant species were accepted for adult feeding although sustained feeding (≥5% leaf area removed) only occurred on 13 species (Table 1). The percentage of leaf area consumed varied among the 13 species receiving sustained feeding damage ($H = 43.5$; df = 12; $P <$

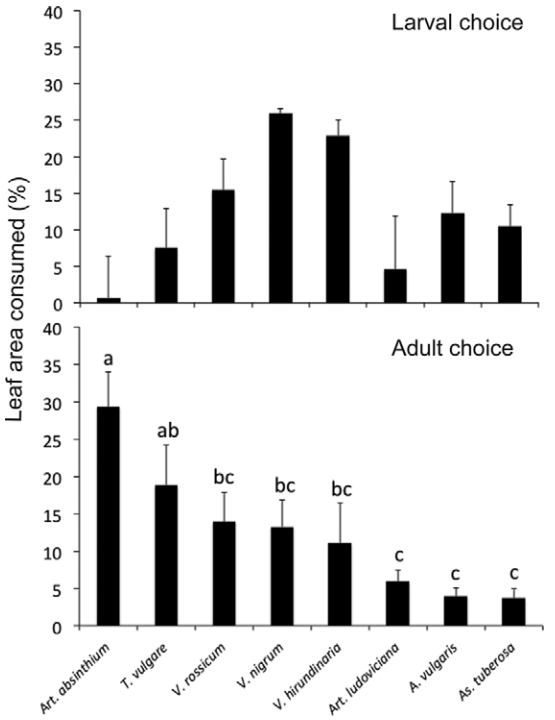

Fig. 1. Relative percentage (mean ± SE) of total leaf area consumed by larvae and adults of *Chrysolina a. asclepiadis* under multiple-choice conditions. Different letters indicate significant differences at $\alpha = 0.05$ (Hotelling's T^2 test).

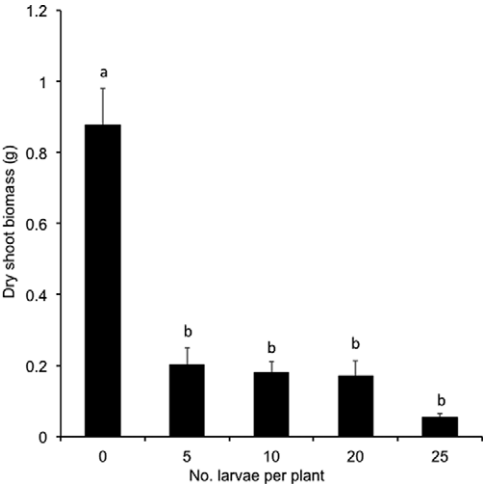

Fig. 2. Effect of *Chrysolina a. asclepiadis* larval density on aboveground biomass (mean ± SE). Different letters indicate significant differences at $\alpha = 0.05$ (Tukey's honestly significant difference (HSD) test).

0.001) (Table 1). Of these species, *As. tuberosa* and *Vincetoxicum* spp. received the greatest feeding damage, but the level of damage to these plants was statistically similar to six other species (Table 1). In oviposition tests, females produced eggs on six of the nine host plant species supporting complete larval development—all species non-native to North America (Table 1). This test confirmed the ability of *C. a. asclepiadis* to feed and lay eggs on *Vincetoxicum* spp., *Art. absinthium*, *Art. vulgaris*, and *T. vulgare.* Subsequent tests confirmed that eggs produced on these plant species were fertile.

Multiple-choice Larval and Adult Feeding. Although there was significant variation in leaf area among plant species exposed to larvae ($F = 13.21$; df = 7, 79; $P < 0.001$) and adults ($F = 3.00$; df = 7, 79; $P = 0.008$) at the beginning of the trials, the proportion of leaf area consumed of each species was not related to the initial leaf area exposed to insects (all Pearson correlations $P > 0.05$). Larvae and adults displayed dissimilar preferences among host species (Fig. 1). Larvae did not prefer *Vincetoxicum* to other host plants ($T^2 = 120.03$; df = 7, 3; $P = 0.090$) (Fig. 1). For adults, feeding on *Vincetoxicum* spp. fell within the middle of the preference hierarchy, but damage to these species was similar to the North American natives *Art. ludoviciana* and *As. tuberosa* ($T^2 = 248.8$; df = 7, 3; $P = 0.034$) (Fig. 1).

Impact of Larval Feeding on Performance of *V. nigrum*. Feeding by five larvae per plant removed 85.4 ± 7.4% (mean ± SE; $n = 9$) of leaf tissue by the end of the experiment and 10, 20, and 25 larvae per plant removed 92.9 ± 7.7%, 95.5 ± 4.5, and 100% of leaf area, respectively ($H = 32.9$; df = 4; $P < 0.001$). This damage resulted in significant reductions to aboveground biomass ($F = 12.78$; df = 4, 44; $P < 0.001$) where damage by five larvae per plant caused an 80% reduction in biomass compared with control plants (Fig. 2). There was no difference in aboveground biomass reduction among densities of 5, 10, 20, and 25 larvae per plant, which caused an 86% reduction in biomass compared with control plants (Fig. 2). Maximum shoot height was significantly affected by larval density ($F = 7.92$; df = 4, 44; $P < 0.001$). Shoot height of control plants increased weekly throughout the 28 d experiment, whereas shoot height of infested plants remained largely unchanged after 7 d ($F = 2.20$; df = 12, 40; $P = 0.031$) (Fig. 3A). By the end of the experiment, relative shoot growth was reduced by 83% on infested plants compared with control plants ($F = 30.6$; df = 4, 44; $P < 0.001$) (Fig. 3B).

Axillary shoot production ($H = 12.91$; df = 4; $P = 0.012$) and the total length of axillary growth were affected by larval feeding ($H = 14.70$; df = 4; $P = 0.005$) (Fig. 4). Control plants never produced axillary growth during the experiment. Plants tended to increase production of axillary shoots with increasing larval density, but not at the highest density (25 larvae per plant) presumably because intense attack killed axillary buds (Fig. 4A). Larvae readily consumed new axillary growth, and total compensatory growth decreased with increasing larval density (Fig. 4B). Finally, the proportion of plants flowering ($H = 20.84$; df = 4; $P < 0.001$) and the number of flowers produced per plant ($H = 22.59$; df = 4; $P < 0.001$) declined with larval density. By the end of the experiment all control

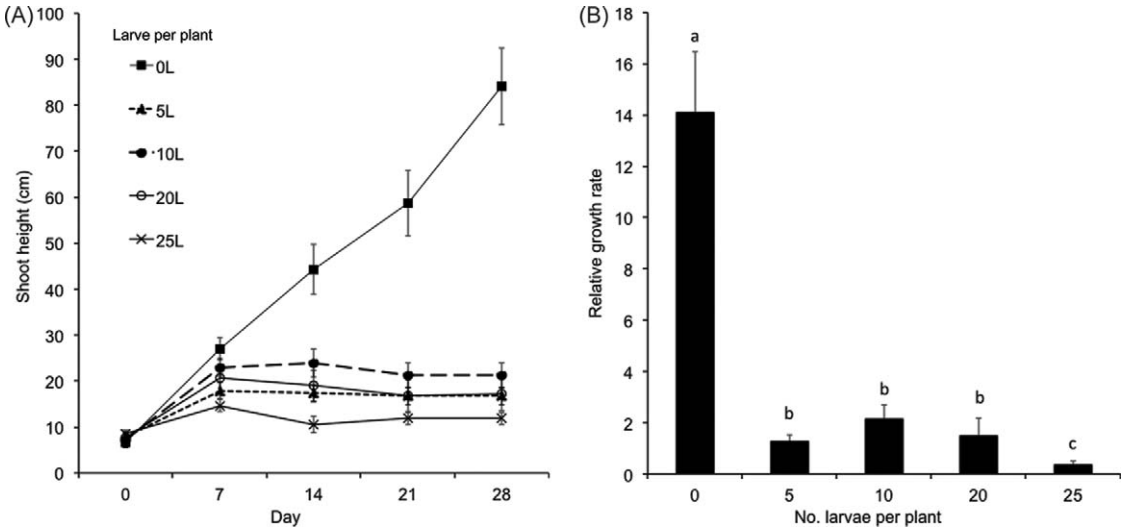

Fig. 3. Effect of *Chrysolina a. asclepiadis* larval density on (A) maximum weekly shoot height (mean ± SE) and (B) relative growth rate (mean ± SE) over the experiment. Different letters indicate significant differences at $\alpha = 0.05$ (Tukey's HSD test).

plants were flowering compared with 44, 11, 22, and 11% of plants that were infested with 5, 10, 20, and 25 larvae per plant, respectively. No developing seedpods were observed on infested plants while maturing seedpods were observed on 44% of control plants.

Discussion

Nine plant species from two families supported successful larval development of *C. a. asclepiadis* in the laboratory under no-choice conditions. Survival was greatest on *Vincetoxicum* spp. (80%), although the Eurasian plants *Art. absinthium*, *Art. vulgaris*, *and T. vulgare* were very suitable hosts for larval development (34–60%). Larvae also successfully developed on three native North American species, but survival was lower and took roughly 9 d longer to complete development on these hosts compared with the Eurasian species. The widely-distributed North American species *Art. ludoviciana* (white sagebrush) was the most suitable host (24%) followed by *As. tuberosa*, and *Art. caudata.*

Exploratory adult feeding was observed on 30 plant species from four plant families. However, sustained

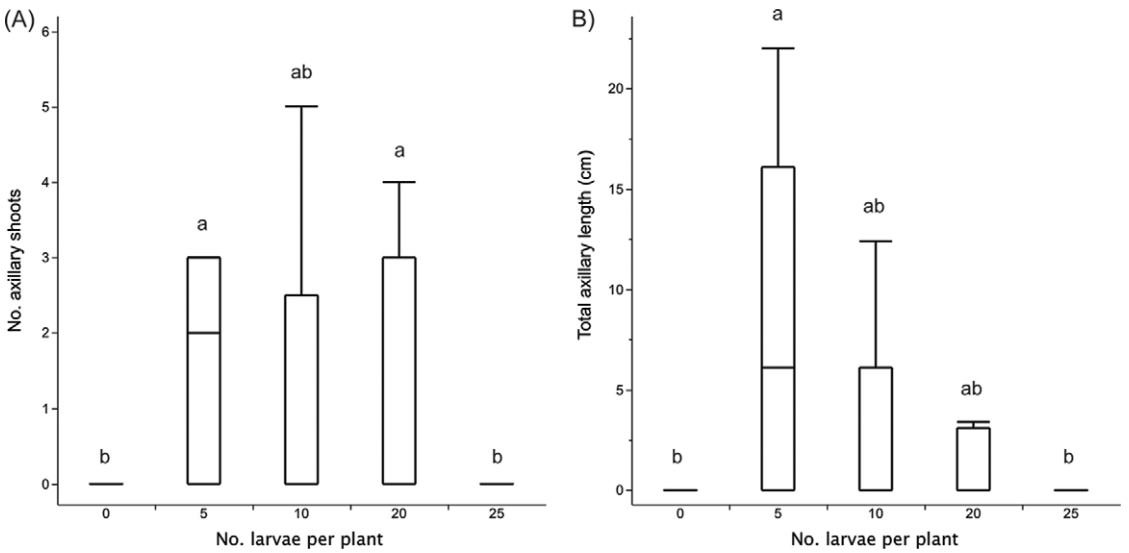

Fig. 4. Effect of *Chrysolina a. asclepiadis* larval density on (A) the number and (B) growth of axillary shoots. The boxes represent the 25th and 75th percentiles about the median and the bars extend to the 10% and 90% values. Different letters indicate significant differences at $\alpha = 0.05$ (Dunn's multiple comparison of ranks test).

feeding by *C. a. asclepiadis* was restricted to 13 species from the Asteraceae and Apocynaceae and feeding damage generally was more severe on species that support complete larval development similar to *C. picturata* (Clark) (Adair and Scott 1997). In oviposition trials, adult *C. a. asclepiadis* laid eggs exclusively when confined to the Eurasian hosts *Vincetoxicum* spp., *Art. absinthium*, *Art. vulgaris*, and *T. vulgare.* No eggs were laid when confined to the North American native species *Art. caudata*, *Art. ludioviciana*, and *As. tuberosa.*

In multiple-choice tests, there was no detectable larval preference among hosts, whereas adults demonstrated a preference for *Art. absinthium* and *T. vulgare* to *Art. vulgaris*, *As. tuberosa*, and *Art. ludoviciana.* Importantly, neither stage avoided the North American species *Art. ludoviciana* and *As. tuberosa* in the presence of *Vincetoxicum.* The lack of a clear signal in feeding preference supports our conclusions from the no-choice tests suggesting that *C. a. asclepiadis* is polyphagous. Under our testing conditions it was apparent that most species are attractive to at least one stage of *C. a. asclepiadis*, although limited adult feeding (<10%) to *Art. ludoviciana*, *Art. vulgaris*, and *As. tuberosa* may indicate that these species possess mild feeding deterrents to this beetle (Rizza and Pecora 1984).

Our host range testing results indicate that *C. a. asclepiadis* is polyphagous and the North American species *Art. caudata*, *Art. ludioviciana*, and *As. tuberosa* are at risk of attack from this beetle. We are uncertain whether *C. a. asclepiadis* will be able to sustain populations in the field on these hosts because of lack of oviposition observed in the laboratory, but 24% of beetles survived to pupation on *Art. ludoviciana.* In contrast, *C. a. asclepiadis* may be able to sustain populations on the Eurasian Asteraceae *Art. absinthium*, *Art. vulgaris*, and *T. vulgare*, which are considered noxious weeds in western North America (USDA-NRCS 2011). Each of these plant species is attacked by closely related subspecies within the *C. aurichalcea* complex (Kippenberg and Döberl 1994, Bieńkowski 1998), which is further evidence of the close ancestral affiliation between *Chrysolina* and Asteraceae.

The genus *Chrysolina* contains nearly 450 described species reported to attack eight plant families (Jolivet and Petitpierre 1976, Bourdoné and Doguet 1991, Bieńkowski 1998). Many *Chrysolina* are considered to be specialized feeders and typically attack species within the same plant genus (Holloway 1964) or family (Jolivet and Petitpierre 1976, Garin et al. 1999). For example, the biological control agents *C. quadrigemina* (Suffrain) (Holloway 1964, Andres 1985) and *C. picturata* (Adair and Scott 1997) are specific at the generic level, whereas *C. polita* (L.) attacks four genera within the Lamiaceae (Jolivet and Petitpierre 1976). Our results and those of others (Hayashi et al. 1994, Kippenberg and Döberl 1994, Bieńkowski 1998) suggest that *C. aurichalcea* s. str. use a diverse array of host plant genera throughout their geographic range, but mainly limited to species within Asteraceae. The only subspecies apparently using non-Asteraceae are *C. a. asclepiadis* and *C. a. bohemica.* The polyphagous habit of these two species bears particular importance on our current view of the evolutionary history of host plant utilization by *Chrysolina* because only one polyphagous clade was thought to exist within *Chrysolina* (no species of *C. aurichalcea* were analyzed in this study) (Garin et al. 1999).

The adoption of chemically dissimilar host plants by *C. a. asclepiadis* and *C. a. bohemica* is not unusual in the Chrysomelidae (Dobler et al. 1996, Mardulyn et al. 1997) although diversification of many herbivores is closely tied to secondary chemistry and host taxonomy (Farrell and Sequeira 2004). We are unaware of why *C. a. asclepiadis* and *C. a. bohemica* have incorporated *Vincetoxicum* into their diet, but physiological, behavioral, and ecological factors are important determinants of host plant specialization (Jaenike 1990, Dobler et al. 1996, Mardulyn et al. 1997). Further biological and molecular study is needed to characterize the population genetics and variation in host plant use throughout the geographical range of these species.

The effect of larval density on *V. nigrum* growth was not linear. Larval feeding damage by *C. a. asclepiadis* was very destructive at low densities where five larvae per plant substantially reduced growth, biomass, and delayed reproduction of *V. nigrum.* A similar reduction in growth and biomass occurred on plants exposed to 10 and 20 larvae per plant despite removal of more plant tissue at these densities. However, the potential impact by *C. a. asclepiadis* needs to be considered in the appropriate context—the effect of larval density expressed per shoot and not per individual plant. This is an important distinction because the impact of leaf feeding to *V. nigrum* varies with plant size (Weed and Casagrande 2010, Weed et al. 2011a) and plant size varies considerably among individuals because of age and site conditions (Milbrath 2008, Weed and Casagrande 2010). Moreover, the impact of *C. a. asclepiadis* feeding on *V. nigrum* performance may be influenced by the synchronization of adult and larval attack with susceptible plant demographic processes as observed with biological control of *H. perforatum* by *C. quadrigemina* in North America (Campbell and McCaffrey 1991, Coombs et al. 2004).

This study indicates that larval feeding damage by *C. a. asclepiadis* has the capability to reduce the vigor and fitness of *V. nigrum* in North America, however, this beetle may attack plant species outside the genus and family of the target weed. Specifically, there is a risk of nontarget feeding to *As. tuberosa* (butterfly weed), which is sold commercially as a garden plant in North America and is considered threatened and vulnerable in five states (Milbrath and Biazzo 2007, USDA-NRCS 2011). In addition, there may be risk of feeding to *Artemisia* spp. native to North America. There are four additional agents under consideration for the biological control of *Vincetoxicum* (Weed et al. 2011b). Among these, the leaf-feeding noctuids *Abrostola asclepiadis* (Denis and Schiffermüller) and *Hypena opulenta* (Christoph) look most promising be-

cause they only attack members of the genus *Vincetoxicum* (Weed 2010a, Hazelhurst 2011).

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