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Host Specificity Testing of *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae) on Wild and Ornamental Plant Species

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***Galerucella calmariensis* has been widely distributed in North America for biological control of purple loosestrife, *Lythrum salicaria*. We tested the host specificity of *G. calmariensis* against 40 nontarget species in 14 previously untested families, determining the ability of *G. calmariensis* adults to survive, feed, produce eggs, and oviposit on these plants in no-choice tests. When adult feeding occurred, we then tested the potential for adult and larval impacts in a series of choice and no-choice tests. For nontarget plants that may co-occur with *L. salicaria*, we also tested the propensity for larval and adult attack under conditions of high *G. calmariensis* density. In no-choice adult feeding tests, *G. calmariensis* did not feed or survive on most of the species tested. Minor damage was observed on *Vernonia fasiculata* (Asteraceae) and five members of the Rosaceae, subfamily Rosoideae, *Fragaria x. ananassa, Filipendula rubra, Rosa setigera, Alchemilla mollis,* and *Rubus idaeus*. In subsequent choice tests, oviposition never occurred on nontargets and no-choice larval establishment tests showed that neonates could not establish on any of these species. For *F. rubra*, which may potentially co-occur with *L. salicaria*, no-choice larval transfer trials showed that third instar *G. calmariensis* larvae did not feed on *F. rubra* and pupated at significantly reduced weights. In a large-cage choice study with larvae and adults, no feeding or oviposition on *F. rubra* occurred. These data support preintroduction host specificity results indicating that normal feeding, oviposition, and development of *G. calmariensis* is confined to *L. salicaria*. However, recent field observations confirm the greenhouse studies indicating that transient feeding by teneral adults may occur on some nontargets under no-choice conditions.** © 2000 Academic Press

***Key Words:* nontarget impacts; host specificity; *Galerucella calmariensis;* weed biological control; risk assessment.**

INTRODUCTION

Increased concern regarding potential impacts on nontarget species has become a major issue in biological control of weeds (McFadyen, 1998). Prior to introduction of a weed biological control agent, host specificity testing must be conducted to make informed decisions about the agent's likely host range and potential for nontarget impacts (Harris, 1993; McEvoy, 1996). These procedures have generally been effective in defining the realized host range of natural enemies released for biological control of weeds. It has been reported that, where postrelease nontarget impacts have been documented, they are generally slow to emerge, are isolated in time and space, and could have been predicted based on host specificity testing (McEvoy, 1996). However, one recent example shows that while some nontarget effects were predicted, others were not or were not considered important at the time of release (Louda *et al.*, 1997).

Purple loosestrife, *Lythrum salicaria* L. (Lythraceae), is a Eurasian plant species accidentally imported into North America in the early 1800s. It has become an invasive species in North America, capable of developing almost monospecific stands in wetlands and other moist habitats (Thompson *et al.*, 1987). Cultural and chemical methods of managing *L. salicaria* have been tested with varying degrees of success (Mullin, 1998). In its native range, a complex of insect herbivores limits the growth and reproduction of *L. salicaria* (Blossey, 1995c). Since the mid 1980s, efforts at biological control of *L. salicaria* in North America have included identification, evaluation, and importation of selected natural enemies (Malecki *et al.*, 1993). *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae) is one of several insect species that have been widely distributed in North America to help manage *L. salicaria* (Hight *et al.*, 1995).

After overwintering as adults, *G. calmariensis* emerge in the spring to feed, mate, and lay eggs on *L. salicaria* (Blossey *et al.*, 1994). Eggs are laid on stems

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or foliage and newly emerged (neonate) larvae move to shoot tips where they feed within the developing leaf tissue (Lindgren, 1997). Larvae typically feed for several days and molt into the second instar before leaving the shoot tip to feed externally on expanded leaves. Defoliation by third-instar larvae can reach 100%. Damage typically progresses from the apex of the plant toward the base. As foliage is depleted, larvae move down stems to seek additional food and ultimately pupate in litter or soil at the base of the plant. New adults emerge during the summer and feed for a short time on *L. salicaria*. Most adults then enter diapause, although observations indicate that a portion may reproduce, giving rise to a second generation (Landis, personal observation).

Prior to introduction of *G. calmariensis* into North America, three categories of plants were examined in host specificity tests to determine their susceptibility to attack by *G. calmariensis*. Plants tested included (1) North American species taxonomically related to *L. salicaria*, (2) selected plants that occur in the same habitats as *L. salicaria*, and (3) certain crop and ornamental species (Blossey *et al.*, 1994; Kok *et al.*, 1992). These tests showed that normal feeding, oviposition, and development of *G. calmariensis* is confined to *L. salicaria* but that transient adult feeding may occur on selected Lythraceae under no-choice conditions. The objective of our studies was to extend host specificity testing of *G. calmariensis* to include ornamental and wild plants (both native and naturalized) commonly found in Michigan and others parts of the United States.

MATERIALS AND METHODS

Plants. Plants selected for host specificity tests represented common garden perennials and wildflowers available in Michigan (Table 1). None of these plants were taxonomically related to *L. salicaria* (Cronquist, 1993). Plants were obtained as either field-grown or potted specimens and transferred to 7.6- to 15.2-liter plastic pots containing potting mix consisting of three parts peat moss and one part potting soil with 0.32 g Osmocote slow-release fertilizer (Scott-Sierra Horticultural Products, Marysville, OH) per liter of soil. Plants were placed in a greenhouse at 28 ± 6°C until adequate foliage developed (10–15 cm minimum plant height). Individual bottom trays were used for daily watering. Plants were acclimated to greenhouse conditions for at least 1 week prior to testing and maintained there until all adult and larval testing was complete.

Insects. Adult *G. calmariensis* were obtained from a colony maintained at Michigan State University. Beetles were F_{1-2} adults reared on *L. salicaria* from overwintered beetles. Newly emerged adults were held on *L. salicaria* foliage for 1–3 days after emergence to assure viability, but not long enough to develop mature eggs. For each plant in the no-choice tests (described below), collections of 10 beetles each were made in the morning and held with detached *L. salicaria* foliage in paper cups prior to transfer to the test plants in the afternoon. No attempt was made to control sex ratio; however, all controls produced large numbers of larvae, indicating that initial sex ratios were not highly biased. In addition to natural daylight (14–16 h), the greenhouse was illuminated with cool-white fluorescent bulbs from 06:00 to 22:00 h providing a minimum light intensity of ca. 1000 lux. All experiments below were conducted between June and September, 1998.

Adult no-choice tests. Adult feeding, oviposition, and survival, as well as larval establishment and survival for *G. calmariensis* were simultaneously evaluated in no-choice tests. *L. salicaria* was used as the control. For each round of testing, three replicates of *L. salicaria* plus three replicates of 9 to 13 nontarget species were tested simultaneously under greenhouse conditions described above. Prior to testing, damaged foliage was removed from test plants. A four-ring wire tomato cage was placed over each plant and covered with a sleeve cage of white No-See-Um netting (Balson-Hercules, Pawtucket, RI). A group of 10 insects was randomly assigned to each plant and released within the sleeve cage. Seven days later, each plant was examined for signs of *G. calmariensis* feeding and reproduction. Live adults and number of egg masses were counted. A small dot of typewriter correction fluid was applied to the stem adjacent to each egg mass located to prevent repeat counting (Nechols *et al.*, 1996). An estimate of the total leaf tissue removed in the *L. salicaria* controls was considered to constitute normal feeding and used to standardize feeding intensity categories. A typical damaged leaf was selected from *L. salicaria* controls and each nontarget plant and a grid of 2.5-mm^2 graph paper was used to estimate the amount of tissue area removed from each leaf (Kok *et al.*, 1992). The total number of damaged leaves per plant was then multiplied by the amount of tissue removed per representative damaged leaf to determine feeding intensity relative to the control. Feeding intensity on nontarget species was categorized as 0 = no feeding, 1 = 0–25% of control damage, 2 = 26–50% of control, 3 = 51–75% of control, and 4 = more than 75% of control. Adult feeding intensity, oviposition, and survival evaluations were repeated at 14 days postinfestation for all nontarget species. Total foliage consumed (cm^2) after 14 days was determined for nontargets where mean feeding intensity was ≥ 1.

Differences among plant species in adult survival and feeding intensity were determined by analysis of variance, and the presence of statistically significant variation ($P < 0.05$) among all groups was established. Post hoc comparisons between groups were

TABLE 1

Wild and Ornamental Plants Used in Host Specificity Tests of *Galerucella calmariensis*, 1998

Family	Species (common name)	Native[a]
Lythraceae	*Lythrum salicaria* L. (purple loosestrife)[b]	
Asclepiadaceae	*Asclepias tuberosa* L. (butterfly weed)	+
Asteraceae	*Aster novae-angliae* L. (New England aster)	+
	Aster umbellatus P. Mill. (flat top aster)	+
	Chrysanthemum x superbum Bergmans ex J. Ingram 'Snow Lady' (Shasta daisy)	
	Coreopsis lanceolata L. (tickseed)	+
	Echinacea purpurea (L.) Moench. (purple coneflower)	+
	Eupatorium purpureum L. (sweet joepyeweed)	+
	Gaillardia x grandiflora Van Houtte 'Goblin' (blanketflower)	
	Helenium autumnale L. (sneezeweed)	+
	Heliopsis helianthoides var. scabra (Dunal) Fern. 'Summer Sun' (false sunflower)	
	Liatris aspera L. (rough blazing star)	+
	Rudbeckia fulgida Ait. 'Goldsturm' (black-eyed Susan)	
	Vernonia fasciculata (Western ironweed)	(+)
Brassicaceae	*Aubrietia deltoidea* (L.) DC. (false rock cress)	
Campanulaceae	*Campanula persicifolia* L. (peachleaf bellflower)	
	Lobelia cardinalis L. (cardinal flower)	+
	Platycodon grandiflorum (Jacq.) DC. (balloon flower)	
Caryophyllaceae	*Dianthus deltoides* L. (maiden pink)	
Euphorbiaceae	*Euphorbia epithymoides* L. (spurge)	
Fabaceae	*Baptisia australis* (L.) R.Br. (wild indigo)	(+)
	Lupinus polyphyllus Lindl. 'Russell hybrids' (lupine)	
Gentianaceae	*Gentiana andrewsii* Griseb. (bottle gentian)	+
Iridaceae	*Iris germanica* L. (tall bearded iris)	
	Iris versicolor L. (blue flag iris)	+
Lamiaceae	*Monarda didyma* L. (bee balm)	(+)
	Physostegia virginiana (L.) Benth. (obedient plant)	+
	Salvia x superba Stapf (sage)	
Polemoniaceae	*Phlox paniculata* L. (phlox)	(+)
Ranunculaceae	*Aquilegia canadensis var. hybrida* Hook. 'McKana Giant' (columbine)	
Rosaceae		
-Rosoideae	*Alchemilla mollis* (Buser) Rothm. (lady's mantle)	
	Filipendula rubra (J. Hill) B. L. Robins. (queen-of-the-pairie)	+
	Fragaria x ananassa Duchesne 'Surecrop' (strawberry)	
	Rosa setigera Michx. 'Blaze' (climbing rose)	
	Rubus idaeus L. 'Nova' (red raspberry)	
-Spiraeoideae	*Aruncus dioicus* (Walt.) Fern. (goatsbeard)	
	Spiraea x bumalda (Burv.) 'Anthony Waterer' (spirea)	
Scrophulariaceae	*Chelone obliqua* L. (turtlehead)	+
	Veronica spicata L. 'Sunny Border Blue' (speedwell)	
	Veronicastrum virginicum (L.) Farw. (Culver's root)	+
Valerianaceae	*Centranthus ruber* (L.) DC. (red valerian)	

[a] +, Michigan native; (+), native to northeastern United States but not to Michigan.
[b] Control.

made using the Student–Newman–Keuls test (SPSS, 1997).

Adult choice tests. For nontargets where feeding intensity was ≥1, adult choice tests were conducted in the greenhouse using potted nontarget species and field-collected *L. salicaria* foliage. The nontarget plant was placed in one quadrant of a 1-m^3 screen cage, and a 500-ml Erlenmeyer flask containing a similar amount of *L. salicaria* foliage was placed in the diagonal quadrant. Five mating pairs of *G. calmariensis* that had fed on *L. salicaria* for 1–2 weeks (i.e., competent for oviposition) were introduced in the center of each cage. Plants were observed every 24 h and watered if necessary. At 72 h, total foliage consumed (cm^2), number of egg masses, average numbers of eggs per mass, and total number of eggs were determined. Five replications of each nontarget species were conducted and the mean ± SEM was calculated.

Larval no-choice tests. For those species tested in adult choice tests, the ability of larvae to feed and develop on nontarget foliage was also evaluated in no-choice feeding assays modified from Blossey *et al.* (1994). A single detached leaf of the test species or *L. salicaria* (control) was placed on moistened filter paper (0.3 ml distilled water) in 5.5-cm-diameter petri dishes. Five neonate larvae (<24 h old) were transferred onto

TABLE 2

Survival, Feeding Intensity, and Oviposition by *Galerucella calmariensis* Adults in No-Choice Tests with Wild and Ornamental Plants, 1998

Family	Species[a]	Adult survival[b]		Adult feeding intensity[c]		Oviposition[d]
		Day 7	Day 14	Day 7	Day 14	
Lythraceae	*Lythrum salicaria* (control)	5.9 ± 1.4	3.7 ± 2.1	4.0 ± 0.0	4.0 ± 0.0	+
Asteraceae	*Aster umbellatus*	2.0 ± 1.7*	0.7 ± 1.2**	0.0**	0.0**	−
	Coreopsis lanceolata	3.0 ± 2.6 ns	1.3 ± 2.3 ns	0.0**	0.0**	−
	Vernonia fasciculata	2.3 ± 2.1*	0.7 ± 1.2**	0.7 ± 0.6**	0.7 ± 0.6**	−
Rosaceae						
-Rosoideae	*Alchemilla mollis*	1.3 ± 1.5**	0.0**	1.0 ± 0.0**	1.0 ± 0.0**	−
	Filipendula rubra	5.3 ± 2.1 ns	2.7 ± 2.1 ns	1.7 ± 0.6**	1.3 ± 0.6**	−
	Fragaria x ananassa	5.7 ± 1.2 ns	4.3 ± 0.6 ns	2.0 ± 0.0**	1.7 ± 0.6**	−
	Rosa setigera	3.3 ± 2.3 ns	0.7 ± 1.2**	1.7 ± 0.6**	1.0 ± 0.0**	−
	Rubus idaeus	1.0 ± 1.7**	0.0**	1.0 ± 1.0**	0.7 ± 0.6*	−

Note. Asterisks indicates that values in the column are significantly different from *L. salicaria* ($P < 0.05$) or **($P < 0.01$) by Student–Neuman–Keuls means separation.

[a] All other species had no survival (day 14) and no feeding (day 7 or 14); $n = 4$ replicates.

[b] Number of live beetles (mean ± SEM).

[c] Feeding intensity of adult *Galerucella* on nontarget species relative to feeding on purple loosestrife controls; 0 = no feeding; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = >75% of feeding on *L. salicaria* controls. Data are expressed as mean feeding intensity ± SEM.

[d] +, Eggs were present on day 14; −, no eggs present.

each leaf using a fine-tipped brush. Dishes were placed in a sealed plastic container and held at 27 ± 1°C. For *Filipendula rubra* (J. Hill) B. L. Robins, 20 dishes (n = 100 larvae) were tested, while for subsequent cases, dishes were replicated five times (n = 25 larvae) for each plant tested. Larval survival, stadia, and feeding damage were evaluated at 24, 48, and 72 h. Larval damage was categorized as 0 = no feeding, (+) = nibbling, + = slight to moderate, and ++ = normal, as per Blossey *et al.* (1994). Larval damage ratings and mean number of larvae surviving to 72 h were contrasted to the control.

F. rubra, which potentially co-occurs with *L. salicaria*, was fed on by adult *G. calmariensis* in the initial no-choice test. Therefore, additional tests were conducted to determine whether *G. calmariensis* larvae could successfully develop on *F. rubra*. In one test, first-instar *G. calmariensis* that had fed on *L. salicaria* for up to 48 h were transferred onto *F. rubra* and *L. salicaria* (control) foliage using the methods described above. Single dishes containing five larvae were replicated five times (n = 25 larvae), with larval survival, stadia, and feeding damage evaluated daily until each individual died or emerged as an adult. Foliage was changed every 72 h or more frequently if required to maintain adequate food.

In a second test, third-instar larvae that had developed on *L. salicaria* were transferred onto *F. rubra* foliage. At the outset, larvae were classified as small (0.003–0.005 mg), medium (0.005–0.007 mg), or large (0.007–0.009 mg) third instars. Three replicates of each size class (five larvae per replicate) were established on both *F. rubra* and *L. salicaria* (control). Larval survival, stadia, and feeding damage were evaluated daily until each individual had pupated and then every 3 days until all individuals had died or emerged as adults. Pupae were weighed on day 11 of the experiment, and pupal weights for test and control plants within initial size groups were contrasted by t tests (SAS Institute, 1996).

Large-cage study. An outdoor 2 × 2 × 2 m cage used for mass rearing of *G. calmariensis* was used for this test. The cage contained 10 *L. salicaria* plants on which large numbers of eggs, larvae, and adults were present. The experiment was initiated just before plants were expected to undergo rapid defoliation similar to that observed in the field under conditions of high *G. calmariensis* population density. The majority of the larval population was in the second and third instars and the foliage had extensive larval damage (upper one-third of canopy was 80–100% defoliated). On day 1 of the test, 20 randomly selected leaves from the lower one-third of the canopy contained an average of 2.5 ± 0.8 (mean ± SEM) larvae per leaf. In addition, 1-min timed counts (n = 3) of teneral adults in the cage averaged 29.7 ± 1.5 (mean ± SEM). The number of teneral adults increased on day 2 of the test (64.0 ± 4.2), indicating continuing adult emergence.

Three *F. rubra* plants in 1-liter pots were selected and trimmed of dead or damaged foliage. Each plant was paired with a flask containing a similar amount of *L. salicaria* foliage. Pairs of flasks and pots were placed into the rearing cage so that the foliage of the test plants was intermingled with the lower one-third of the *L. salicaria* canopy containing *G. calmariensis* larvae.

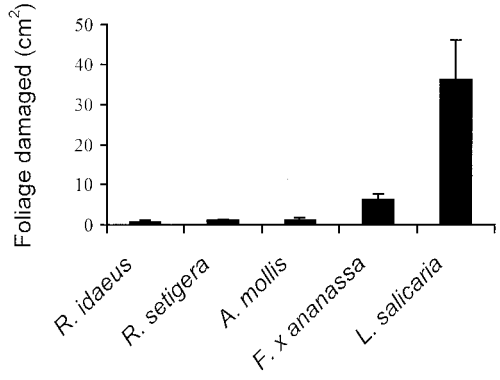

FIG. 1. Mean ± SEM foliage damaged by *Galerucella calmariensis* adults in 14 days in no-choice feeding trials, 1998.

Three pairs (replicates) of the test plant and control were randomly assigned to different areas of the rearing cage. At 2, 4, and 6 days after placement, *L. salicaria* test foliage and *F. rubra* plants were removed and the number of adults and larvae on each counted. The number of leaves with adult and larval damage was also recorded. The *F. rubra* plants were replaced in the cage. New *L. salicaria* foliage was placed in the flasks on days 2 and 4.

RESULTS

Adult no-choice tests. At day 7, live *G. calmariensis* adults could be found on most nontarget species. However, by day 14, only 6 of the 40 species tested had live adults (Table 2). Adult survival was highest on *L. salicaria, Fragaria x ananassa,* and *Filipendula rubra.* Adult feeding on nontarget species was confined to *Vernonia fasiculata* (Asteraceae) and the Rosaceae, subfamily Rosoideae, *Alchemilla mollis, F. rubra, F. x. ananassa, Rosa setigera,* and *Rubus idaeus* (Table 2). Feeding was highest on *F. x. ananassa* and *F. rubra;* however, the feeding intensity never exceeded a mean value of 2 (i.e., 26–50% of control feeding) (Table 2). After 14 days, the maximum amount of foliage eaten was less than 6.3 cm^2 for any nontarget species, in contrast to a mean of 36 cm^2 removed in the *L. salicaria* controls (Fig. 1). Oviposition, larval development, and adult emergence were observed only on *L. salicaria.*

Adult choice tests. In choice tests, *G. calmariensis* adults did not damage *F. rubra, F. x ananassa,* and *R. idaeus,* (Table 3). On *A. mollis* and *R. setigera* limited damage occurred in certain replicates. Damage to *A. mollis* totaled 0.09 cm^2 of foliage removed and occurred in just one of the five replicates. On *R. setigera* damage occurred in three of five replicates and resulted in 0.03, 0.09, and 1.2 cm^2 of foliage removed, while damage to each control plant exceeded 30 cm^2. Oviposition did not occur on any nontarget species. Egg numbers on *L. salicaria* averaged 25 to 55 masses/control. The mean egg clutch sizes of 5.8–7.8 eggs per mass on *L. salicaria* controls (Table 3) were similar to (Lindgren, 1997) or slightly larger than (Blossey, 1995a) values previously reported.

Larval no-choice tests. Neonate larvae were unable to develop and survive on any nontarget plant species (Table 4). Survival on *L. salicaria* averaged 84% in two tests; however, the test with *A. mollis, R. setigera,* and *R. idaeus* should be interpreted with caution due to significant control mortality (68%) in the first 24 h. During the same period, mortality in the nontargets ranged from 44 to 88%. In the subsequent 48 h, control mortality was low (6%) versus 100% for each nontarget. Transfer of previously fed first-instar larvae to *F. rubra* resulted in limited development (12.5% of individuals); however, these larvae consumed little, matured slowly, and either were unable to pupate or died before eclosion (Table 5). Irrespective of initial size class, third-instar larvae transferred from *L. salicaria*

TABLE 3

Feeding Damage and Oviposition by *Galerucella calmariensis* Adults in 72-h Choice Tests, 1998

Plant	*n*	Adult feeding[a]	Number of egg masses (mean ± SEM)	Number of eggs/mass (mean ± SEM)	Total eggs (mean ± SEM)
Filipendula rubra versus	5	0	0	0	0
Lythrum salicaria	5	++	37.0 ± 2.0	5.8 ± 0.5	218 ± 27.5
Fragaria x. ananassa versus	5	0	0	0	0
Lythrum salicaria	5	++	55.0 ± 9.9	6.7 ± 0.4	361 ± 52.6
Alchemilla mollis versus	5	(+)	0	0	0
Lythrum salicaria	5	++	51.8 ± 9.9	7.8 ± 0.9	377 ± 58.2
Rosa setigera versus	5	(+)	0	0	0
Lythrum salicaria	5	++	39.4 ± 8.9	6.2 ± 0.4	237 ± 50.0
Rubus idaeus versus	5	0	0	0	0
Lythrum salicaria	5	++	25.0 ± 6.2	6.1 ± 0.6	165 ± 46.8

[a] 0 = no feeding; (+) = occasional nibbling; + = slight to moderate feeding; ++ = normal feeding equivalent to *L. salicaria* controls.

TABLE 4

Survival and Feeding Damage by *Galerucella calmariensis* Neonates in 72-h Larval No-choice Tests, 1998

Plant	*n*	Number alive per dish (mean ± SEM)	Larval feeding damage[b] per dish
Filipendula rubra	20	0	0
Lythrum salicaria	20	4.2 ± 0.5	++
Fragaria x. ananassa	5	0	0
Lythrum salicaria	5	4.2 ± 0.4	++
Alchemilla mollis	5	0	0
Rosa setigera	5	0	0
Rubus idaeus	5	0	0
Lythrum salicaria	5	1.4 ± 0.4	++

[a] *n* = number of dishes each containing five larvae.
[b] 0 = no feeding; (+) = occasional nibbling; + = slight to moderate feeding; ++ = normal feeding equivalent to *L. salicaria* controls.

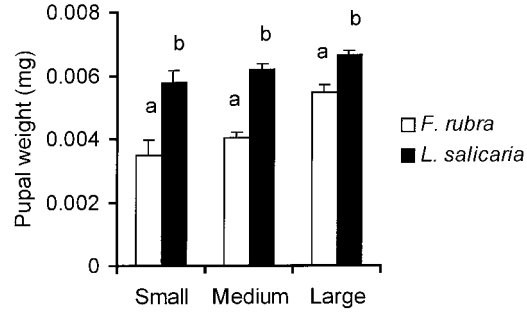

FIG. 2. Mean ± SEM pupal weight of third-instar *Galerucella calmariensis* larvae transferred from *L. salicaria* to *F. rubra*. Small, medium, and large indicate initial larval size class (by weight). Letters indicate the result of *t* test comparisons conducted within each size class, 1998.

to *F. rubra* did not feed and pupated at significantly ($P < 0.05$) lower final weights than those transferred to *L. salicaria* (Fig. 2).

Large-cage study. Over 6 days of the test, no feeding occurred on *F. rubra* in the large-cage study (Table 6). During this time defoliation of the original *L. salicaria* foliage was estimated to have exceeded 80%. Control flasks containing *L. salicaria* foliage were heavily attacked. Feeding by late-instar larvae gradually gave way to adult feeding over the duration of the test as most larvae pupated and teneral adults began to emerge (Table 6). This situation parallels that which occurs in the field. While adults and larvae of *G. calmariensis* were observed to rest or crawl on the *F. rubra* plants (Table 6), feeding was never initiated.

DISCUSSION

Manguin *et al.* (1993) stated that evolution within the genus *Galerucella* has occurred primarily by specialization on wetland plants, and several *Galerucella* spp. worldwide are known to include Rosaceae in their host range. In central and northern Finland, *G. sagittariae* (Gyll.) is considered a pest of cloudberry (*Rubus chamaemorus* L.) and cultivated strawberry (*F. x ananassa* cv.) (Hippa and Koponen, 1975, 1984). In North America, *G. stefanssoni* Brown feeds on cloudberry and *G. quebecensis* Brown on marshflower (*Potentilla palustris* (L.) Scop.) (Manguin *et al.*, 1993). In our study, adult *G. calmariensis* also fed to a limited degree on several Rosaceous plants that we tested. Among the nontarget species attacked, *Rosa setigeria* and *A. mollis* are important ornamental plants, whereas *F. x ananassa* (strawberry) and *R. idaeus* (red raspberry) are cultivated crops. The feeding on these plants was of a low intensity in contrast to that on *L. salicaria*. It is also notable that, while these plants are widely grown in Europe, they are not reported to be attacked by the indigenous *G. calmariensis* (B. Blossey, personal communication, Cornell University, Ithaca, NY).

Throughout the tests, *G. calmariensis* never laid eggs on any nontarget species, while large numbers of

TABLE 5

Survival, Feeding Damage, and Development by *Galerucella calmariensis* in a Larval No-Choice Test Using First Instars Previously Fed (48 h) on *L. salicaria*, 1998

		5 Day			10 Day			Final	
Plant	*n*	Number alive	Number per instar	Feeding damage[a]	Number alive	Number per instar	Feeding damage[a]	Earliest pupation (days)	Adults
Filipendula rubra	25	3	1 First 2 Second	+[b]	3	3 Third	+[c]	17[d]	0[e]
Lythrum salicaria	25	24	24 Third	++	23	22 Prepupa 1 Pupa	++	10	5

[a] 0 = no feeding; (+) = occasional nibbling; + = slight to moderate feeding; ++ = normal feeding equivalent to *L. salicaria* controls.
[b] 3–6% of leaf surface removed in *F. rubra* versus 50–80% in *L. salicaria*.
[c] 8–12% of leaf surface removed in *F. rubra* versus 0% in *L. salicaria* as all insects had ceased feeding.
[d] Died in an incomplete molt to pupal stage.
[e] One individual escaped and the last pupated (ca. $\frac{1}{3}$ normal size) but died before emergence.

TABLE 6

Galerucella calmariensis Adult and Larval Numbers and Feeding Damage on *L. salicaria* and *F. rubra* in an Outdoor Large-Cage Study Mimicking Conditions in a *L. salicaria* Stand Heavily Attacked by *G. calmariensis*

Time period and species	*n*	Adult-damaged leaves (mean ± SEM)	Larval-damaged leaves (mean ± SEM)	Number of larvae[a] (mean ± SEM)
2 Day				
Filipenduala rubra	3	0	0	1.5 ± 0.9
Lythrum salicaria	3	18.3 ± 2.3	62.3 ± 5.2	68.7 ± 3.5
4 Day				
Filipenduala rubra	3	0	0	0.7 ± 0.3
Lythrum salicaria	3	51.3 ± 7.9	48.3 ± 15.7	27.7 ± 3.7
6 Day				
Filipenduala rubra	3	0	0	0
Lythrum salicaria	3	84.3 ± 18.5	12.7 ± 5.8	5.0 ± 1.5

[a] Larvae observed to crawl and rest on *F. rubra* but not feed.

eggs were laid on *L. salicaria* controls in both no-choice and choice tests. Our studies revealed that, when placed on a nontarget species, neonate larvae were not able to survive, while a low percentage of previously fed first instars survived for some time but were unable to complete development. Third-instar *G. calmariensis* larvae would not feed on *F. rubra* in no-choice tests. The same was true for the more realistic large-cage study involving both larvae and teneral adults. We thus conclude that these nontargets are not within the physiological host range of *G. calmariensis.* These findings also indicate that several steps in *G. calmariensis*' host selection process (adult feeding, oviposition, larval survival, and development) are generally not completed, suggesting that future adaptation to these plants may be difficult.

One risk that was identified by these tests is the potential for limited adult feeding by *G. calmariensis* on nontarget hosts under no-choice conditions. Previous field tests have shown that when large numbers of teneral adult *G. calmariensis* and *G. pusilla* emerge in a site where *L. salicaria* is completely defoliated (i.e., a no-choice situation), they will feed on *Lythrum alatum* Pursh and *Decodon verticillatus* (L.) Ell. (Lythraceae), resulting in up to 30–40% defoliation (Blossey *et al.*, 1994). Similar phenomena have recently been observed in Rhode Island (1999), where large numbers of *G. calmariensis* emerging from a site of 100% *L. salicaria* defoliation were observed to defoliate several *Rosa multiflora* Murray (Rosaceae) plants, one shoot of *Salix discolor* Muhl. (Salicaceae), and one leaf of *Myrica pensylvanica* Loisel. (Myricaceae), while other wetland plants in the vicinity were untouched (E. A. Tewksbury, personal communication, University of Rhode Island, Kingston, RI). One of the authors (Landis, unpublished data) has observed a similar mass emergence of *Galerucella* adults in Michigan at a site where both *G. calmariensis* and *G. pusilla* had been released. No live purple loosestrife foliage remained in the emergence area and hundreds of thousands of beetles were seen resting and dispersing from the site. Several *Potentilla anserina* L. (Rosaceae) plants growing in the middle of a gravel road on top of a dike bordering the site were ca. 60% defoliated by *Galerucella* adults. Individuals of *P. anserina* growing a few meters away on the sides of the dike were untouched and no feeding was evident on the diverse surrounding vegetation. The specimens collected were unfortunately misplaced by a laboratory worker and their identification could not be confirmed. Because the native species *G. quebecensis* is known to feed on *Potentilla* spp., it is uncertain whether this is a case of nontarget feeding. However, in conjunction with the adult no-choice tests, these observations suggest that transient adult feeding in the field may occur on plants that *G. calmariensis* adults will accept under no-choice conditions.

Host specificity testing cannot eliminate the possibility that nontarget effects may occur at some point in the future (McFayden, 1998). The goal of preintroduction host specificity testing is to determine the potential host range of the biocontrol agent so that an informed risk assessment can be performed (Blossey, 1995b; McEvoy, 1996). Prior to its introduction into North America, host specificity testing on 48 nontarget species in 14 families indicated that normal feeding, oviposition, and development of *G. calmariensis* was confined to *L. salicaria* (Blossey *et al.*, 1994). Based on those studies, *G. calmariensis* was determined to be host specific to *L. salicaria.* Our studies support that conclusion and extend the result to 40 additional species in 14 previously untested families. However, our laboratory results, coupled with recent field observations, suggest that transient feeding by teneral adults on selected nontargets may be expected. The conditions under which such feeding may occur in the field and its impact on nontarget populations should be investigated.

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