Establishment of Insect Biological Control Agents from Europe Against *Lythrum salicaria* in North America

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Environ. Entomol. 24(4): 967–977 (1995)

ABSTRACT Three European biological control agents of the exotic, wetland, perennial plant purple loosestrife, *Lythrum salicaria* L., were released in North America in 1992 and 1993. Two leaf-feeding beetles, *Galerucella calmariensis* L. and *G. pusilla* Duftschmidt, from 2 climatically different source populations in Germany, were released in 10 different states and 6 Canadian provinces. The importance for establishment success of climatic preadaptation, number of individuals released, release of laboratory or field-collected material, and confinement of release were investigated in a series of experimental releases. Both *Galerucella* species became established at all 1992 release sites regardless of their origin or release method. Higher survival in cages was found for releases of 600 beetles compared with releases of 200 beetles. The amount of litter, number of standing dead stems, or host-plant density did not affect establishment. A root-feeding weevil, *Hylobius transversovittatus* Goeze, was released in 9 states and 2 Canadian provinces, and established in the field in 6 states and both provinces. The 3 species successfully passed the most critical phase for establishment in North America; production of the generation following release.

KEY WORDS *Galerucella, Hylobius transversovittatus, Lythrum salicaria,* biological control

PURPLE LOOSESTRIFE, *Lythrum salicaria* L. (Lythraceae), a wetland perennial native to Europe, was introduced to North America along the northeastern maritime coast at the beginning of the nineteenth century. It has spread progressively westward and now occurs throughout the northern half of the United States and southern Canada (Stuckey 1980, Thompson et al. 1987). Purple loosestrife aggressively invades wetlands and displaces native vegetation. No effective long-term control technique is available. Short-term control can be achieved by water-level manipulation, mowing or cutting, burning, or herbicide application (Malecki and Rawinski 1985, Thompson et al. 1987). These techniques are labor intensive, costly, and, in the case of herbicides, nonselective and harmful to nontarget vegetation (Skinner et al. 1994).

Current efforts to control *L. salicaria* focus on introducing host-specific phytophagous insects from the native range of the plant in Europe (Hight and Drea 1991, Malecki et al. 1993). European studies identified 3 insects (a root-feeding weevil, *Hylobius transversovittatus* Goeze, and 2 leaf-feeding beetles, *Galerucella calmariensis* L. and *G. pusilla* Duftschmidt) as promising biological control agents (Blossey and Schroeder 1986, 1991; Blossey 1993, 1995). These 3 species are host-specific (Blossey et al. 1994a, b), and their field introduction was approved in 1992[6] by the Animal and Plant Health Inspection Service, USDA, and by the Plant Protection Division of the Food, Production and Inspection Branch, Agriculture Canada. Field releases were initiated in North America in the summer of 1992.

Despite a long history of using insects for control of weeds and the considerable improvement in procedures, only ≈60% of released agents become established (Crawley 1989). Various release methods have been used, but their outcome cannot be predicted. Basic research involving biology, demographics, and ecological interactions of host plants and control agents is lacking (Crawley 1989). Taxonomy and climatic preadaptation of control agents, number of individuals released, numbers and timing of releases, predators, and weather conditions are considered important for establishment (Crawley 1989, Lawton 1990). However, the contributions of these factors lack scien-

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[6]Approval was granted for releases of *H. transversovittatus* in 1991. The releases were experimental in nature and confined to field cages only at the New York and Pennsylvania sites.

0046-225X/95/0967–0977$02.00/0

tific evaluation and are largely observational (Lawton 1990). Our goal in this study of the importance of climatic preadaptation, number of individuals released, and containment of release was to improve and evaluate release procedures for control agents of *L. salicaria*. This article also documents North American releases until the autumn of 1993.

The two *Galerucella* spp., *G. pusilla* and *G. calmariensis* (Coleoptera: Chrysomelidae), share the same life-history features and occupy similar ecological niches (Blossey 1991). Adult beetles are easily distinguished (Manguin et al. 1993), but eggs and larvae of the 2 species are virtually indistinguishable. Adult beetles overwinter in leaf litter and appear in early spring on sprouting plants where they preferentially eat young buds and leaves. Females lay eggs from May through July, with peak oviposition occurring in June. Eggs are laid in batches on stems and leaves, and each egg is covered with a line of frass. Young larvae feed in buds on developing leaves. Later instars eat all plant parts. Pupation occurs in soil or litter beneath the plant. Early emerging adults of the next generation have a short oviposition period in July before overwintering (Blossey 1991).

Adult *H. transversovittatus* (Coleoptera: Curculionidae) overwinter in soil and appear on *L. salicaria* in early spring. Adults are nocturnal and consume foliage and stem tissue. The oviposition period lasts from May to early September, and females produce 3–4 eggs per day during the peak. Eggs are laid into soil close to the host plant or into a stem. Young larvae preferentially feed on the outer layers of larger roots, and later instars mine the rootstock. Mines are filled with light-brown packed frass. Larvae develop over a period of 1–2 yr, depending on the time of oviposition, before forming a pupation chamber in the upper part of the root. Length of adults varies between 5 and 15 mm, depending on food quality. Adults are long-lived and can overwinter several times. Newly emerged beetles have a short oviposition period before overwintering (Blossey 1993).

Materials and Methods

Collection and Shipment. Larval *G. calmariensis* and *G. pusilla* were field collected in June 1992 at 2 European sites; northern (Meggerdorf) and south central (Gelnhausen) Germany (Fig. 1A). To eliminate hymenopterous parasitoids of adults and larvae, shoots with 3rd instars were cut at ground level, held in containers with moist florist foam at the bottom, and covered with gauze and a plastic bag. Under these conditions, the plant material remained fresh for >1 wk, which allowed the larvae to complete their development. Larvae pupated in florist foam or in accumulating litter. Emerging adults were collected with aspirators and kept in rearing cages with fresh food until shipment. Rearing was conducted in a greenhouse under ambient temperatures and photoperiod.

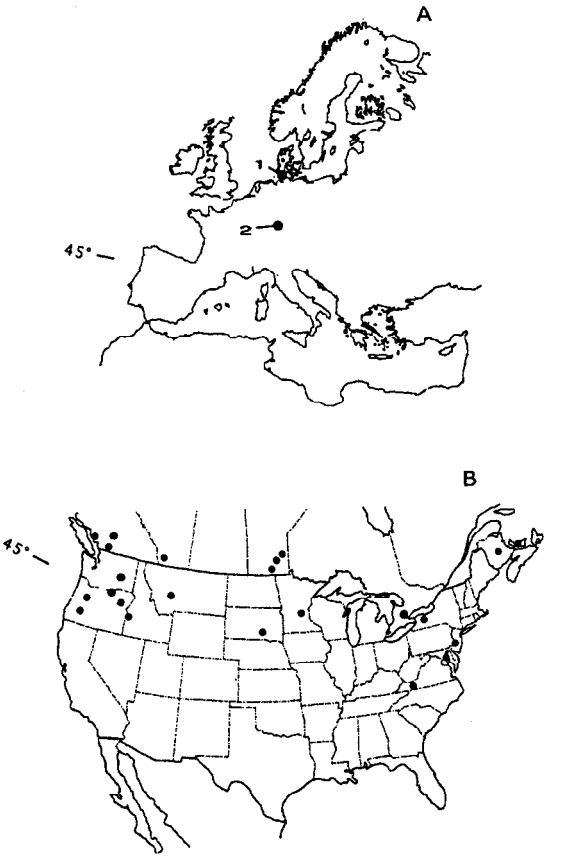

Fig. 1. (A) European collection sites for *Galerucella* spp.: 1, Meggerdorf; 2, Gelnhausen. (B) North American release sites of *Galerucella* spp.

The low abundance of *H. transversovittatus* did not allow field collections of this species for immediate shipment. Parental stock for this colony was collected at various sites in northern and central Europe from 1986 to 1990 (Blossey 1993). The insect was then reared outdoors in potted plants in northern Germany.

For each species, pathogen-free insects were shipped to the quarantine facility at Virginia Polytechnic Institute and State University at Blacksburg, VA, in small containers with cut stems of *L. salicaria* as food. They arrived within 48 h. Insects were kept for ≈1 wk, during which time weak and diseased individuals were separated. Robust individuals were then distributed to the various locations identified as initial release and nursery sites across North America.

Releases. Minimum release-site criteria for initial release of the control agents were developed for the United States. A list of the criteria was sent to potential cooperators, and those sites that met all criteria were chosen for insect releases (Hight and Drea 1991). In 1992, *Galerucella* spp. were released by collaborators in 8 states in the United States and 1 Canadian province (Table 1; Fig. 1B).

Laboratory colonies were initiated in Ontario, Alberta, New York, Virginia, and Washington. Releases into 2 additional states and 5 provinces were made in 1993 (Table 1; Fig. 1B). Adults for these releases were either obtained from Europe (identical collection sites as in 1992) or from laboratory-reared offspring of adults shipped in 1992. In 1993, Canadian releases of *Galerucella* spp. were made in Ontario, Prince Edward Island (PEI), New Brunswick, Manitoba, Alberta, and British Columbia. Releases of the 2 species of *Galerucella* were kept separate from each other in some cases: *G. pusilla* was released in New Brunswick; *G. calmariensis* in Alberta and British Columbia. However, both species were released at some sites in Manitoba and Prince Edward Island.

In 1992, *H. transversovittatus* adults and eggs were released into the field in 7 different states and 2 Canadian provinces (Table 2). Field cage releases had been made in 1991 at the 2 sites in New York and Pennsylvania. The 1992 releases were at the same locations. Nocturnal adults and larvae feeding below the surface make it difficult to estimate weevil abundance at a release site. Dissecting roots to check for larvae was destructive and might have had a substantial negative impact on the establishment and population buildup of *H. transversovittatus*. Therefore, few roots were examined to check for larval feeding damage at the New York, Pennsylvania, Ontario, and Manitoba sites. *H. transversovittatus* colonies for future releases were established in laboratories or gardens in Ontario, Alberta, New York, Virginia, Maryland, Minnesota, Montana, Oregon, Washington, and Colorado. Subsequent releases from rearings were made in 1992 and 1993 (Table 2). The presence of the beetles was periodically checked at all sites.

Experiments. Experiments to study the importance of factors influencing establishment of biological control agents with both *Galerucella* species were conducted in northwestern New York and southeastern Pennsylvania. Both sites are wildlife management areas where purple loosestrife monocultures have been established for at least 5 yr. Vegetation in the study areas was uniform; few plant species were present besides *L. salicaria*. Releases were made into walk-in field cages (Saran Cages, 20 by 20 mesh, Lumite, Gainsville, GA; small, 1.8 by 1.8 by 1.8 m; large, 3.6 by 3.6 by 1.8 m). Cages were erected in July, and beetles were released as recently emerged adults in August. In early November, cage screening was removed for winter storage and replaced before the beetles became active in April 1993.

Climatic Matching. The northern collection site (Meggerdorf, 54° N) is located between the North Sea and the Baltic Sea (Fig. 1A) and experiences reduced temperature fluctuations between winter and summer. Winters are mild and humid with average temperatures between 0 and 5°C. Summers are cool and wet with average temperatures between 15 and 20°C. Annual precipitation is 74 cm (Meteorological Office 1958). The proportion of *G. calmariensis* of the *Galerucella* collected at Meggerdorf was 90%.

The southern collection site (Gelnhausen, 50° N) is located at the northern end of the Rhine valley (Fig. 1A) and has a more continental climate with warmer summers and higher temperature fluctuations between winter and summer. Winters are mild and humid with average temperatures around 5°C. Summers are warm and humid with average temperatures between 20 and 25°C. Annual precipitation is 61 cm (Meteorological Office 1958). The proportion of *G. calmariensis* of the *Galerucella* collected at Gelnhausen was 30%.

Releases in North America were made between 36° and 50° N and across the whole continent (Fig. 1B). At the Pennsylvania site (40° N), average winter temperatures reach 5–10°C and average summer temperatures are 25–30°C. Relative humidity is high, and average annual precipitation is 110 cm (Meteorological Office 1958). At the New York site (43° N), average winter temperatures are below 0°C and average summer temperatures are 20–25°C. Relative humidity is high and average annual precipitation is 97 cm (Meteorological Office 1958).

To evaluate the importance of climatic preadaptation, adults from the 2 source populations were kept separate and released into different field cages at the New York and Pennsylvania sites. Two hundred adults were released into large cages randomly assigned to 1 of 2 treatments. Ten cages (6 in New York and 4 in Pennsylvania) received adult *Galerucella* spp. from Gelnhausen and 15 cages (11 in New York and 4 in Pennsylvania) adult *Galerucella* spp. from Meggerdorf.

Number Released. To study the importance of the number of individuals released to achieve initial establishment, 20, 60, or 180 adults from Gelnhausen were released into each of 12 randomly selected small field cages. In addition, 4 randomly selected large field cages received 600 individuals each and 11 received 200 adults each from the northern Meggerdorf population. These experiments were conducted at the New York site.

Confinement. To evaluate the importance of cage size on establishment, beetles from Gelnhausen involved in the climatic and number experiments were used. Survival in 4 small cages that each received 180 beetles was compared with survival in 6 large cages that each received 200 beetles.

To compare establishment with and without confinement, open field releases were made in close vicinity to the cages. Four hundred beetles from Gelnhausen were released at both the Pennsylvania and New York field sites.

Litter Influence. Both *Galerucella* species overwinter as adults mainly in the leaf litter and hollow stems, but a few individuals were found in old purple loosestrife inflorescences (B. B., personal observation). We tested the hypothesis that the

Table 1. Location, origin, method, and number of stages of *Galerucella* spp. released into North America and their fate regarding establishment

State/Province	County/location	Releases					Established in	
		Origin	No.	Date	Stages	Method	1993	1994
Pennsylvania	Philadelphia	Gelnhausen	560	July 1992	A	Open	Yes	Yes
	Philadelphia	Gelnhausen	800	July 1992	A	Cage	Yes	Yes
	Philadelphia	Meggerdorf	800	Aug. 1992	A	Cage	Yes	Yes
New York	Genese	Gelnhausen	2,216	July 1992	A	Cage	Yes	Yes
	Genese	Gelnhausen	400	July 1992	A	Open	Yes	Yes
	Genese	Meggerdorf	4,350	Aug. 1992	A	Cage	Yes	Yes
	Genese	New York laboratory by way of Guelph	>1,000	June 1993	A, L, P	Cage	—	Yes
Maryland	Prince Georges	Gelnhausen	400	Aug. 1992	A	Open	Yes	Yes
Virginia	Wise	Mix[a]	1,590	Aug. 1992	A	Open	Yes	Yes
Minnesota	Ramsey	Gelnhausen	400	Aug. 1992	A	Open	Yes	Yes
	Ramsey	Gelnhausen	1,700	Aug. 1992	A	Cage	Yes	Yes
	Houston	Gelnhausen/Meggerdorf	1,000	July–Aug. 1993	A	Open	—	Yes
	Winona	Gelnhausen/Meggerdorf	1,000	July–Aug. 1993	A	Open	—	Yes
	Goodhue	Gelnhausen/Meggerdorf	1,000	July–Aug. 1993	A	Open	—	Yes
	Ramsey	Gelnhausen/Meggerdorf	1,000	July–Aug. 1993	A	Open	—	Yes
	Crow Wing	Gelnhausen/Meggerdorf	250	Aug. 1993	A	Open	—	Yes
	Ramsey	Gelnhausen/Meggerdorf	30	Oct. 1993	A	Open	—	Yes
	Washington	Gelnhausen/Meggerdorf	250	Sept. 1993	A	Cage	—	Yes
Washington	Whitman	Gelnhausen	2,520	Aug. 1992	A	Cage	Yes	Yes
	Grant	Gelnhausen	1,680	Aug. 1992	A	Open	Yes	Yes
	Whitman	Gelnhausen	420	Aug. 1992	A	Laboratory	—	—
	Whitman	Gelnhausen	400	July 1993	A	Cage	—	Yes
	Whitman	Gelnhausen	145	July 1993	A	Open	—	Yes
	Grant	Gelnhausen	1,000	July 1993	A	Open	—	Yes
	Grant	Gelnhausen	600	July 1993	A	Open	—	Yes
	Whitman	Meggerdorf	200	Aug. 1993	A	Cage	—	Yes
	Grant	Meggerdorf	800	Aug. 1993	A	Open	—	Yes
	Grant	Meggerdorf	660	Aug. 1993	A	Open	—	Yes
	Whitman	Meggerdorf	200	Aug. 1993	A	Laboratory	—	—
South Dakota	Bonhomme	Gelnhausen	1,000	July 1993	A	Cage	—	?
	Bonhomme	Gelnhausen	1,000	July 1993	A	Cage	—	?
Oregon	Douglas	Gelnhausen	500	Aug. 1992	A	Open	Yes	Yes
	Marion	Gelnhausen	400	Aug. 1992	A	Open	Yes	Yes
	Morrow	Gelnhausen	420	Aug. 1992	A	Cage	Yes	Yes
	Polk	Gelnhausen	1,050	Aug. 1992	A	Cage	Yes	Yes
	Umatilla	Gelnhausen	440	Aug. 1992	A	Open	Yes	Yes
	Union	Gelnhausen	630	Aug. 1992	A	Cage	Yes	Yes
	Yamhill	Gelnhausen	630	Aug. 1992	A	Cage	Yes	Yes
Idaho	Payette	Gelnhausen	420	Aug. 1992	A	Open	Yes	Yes
Montana	Lake	New York	100	June 1993	A	Cage	Yes	Yes
	Gallatin	Meggerdorf	2,150	Aug. 1993	A	Laboratory	—	—
British Columbia	Fraser	Lethbridge	250	June 1993	A	Open	—	Yes
	Fraser	Lethbridge	300	Aug. 1993	A	Cage	—	No
	Gr. Vancouver	Lethbridge	270	June 1993	A	Cage	—	Yes
	Gr. Vancouver	Lethbridge	38	June 1993	A	Cage	—	Yes
	Gr. Vancouver	Lethbridge	350	July 1993	L	Cage	—	No
	Gr. Vancouver	Lethbridge	50	July 1993	A	Cage	—	Yes
	Okanogan-Sim.	Lethbridge	300	July 1993	A	Open	—	No

Table 1. Continued

State/Province	County/location	Releases					Established in	
		Origin	No.	Date	Stages	Method	1993	1994
Alberta	Lethbridge	Lethbridge	388	June 1993	A	Open	—	Yes
Manitoba	St. Clements	Lethbridge	1,726	June–Aug. 1993	A	Cage	—	Yes
	Port. la Prairie	Lethbridge	616	July–Aug. 1993	A	Cage	—	Yes
Prince Edward Island	Queens	Guelph	770	July–Sept. 1993	A	Open/cage	—	No
	Kings	Guelph	320	July 1993	A	Open	—	?
New Brunswick	Queens	Guelph	148	Sept. 1993	A	Open	—	No
Ontario	Wellington	Guelph	2,600	Sept. 1992	L	Open	No	Yes
	Wellington	Guelph	200	Sept. 1992	A	Open	Yes	Yes
	Wentworth	Guelph	1,200	May 1993	A	Open	—	Yes
	Waterloo	Guelph	200	May 1993	A	Open	—	Yes
	Waterloo	Guelph	4,100	June 1993	A	Open	—	Yes
	Waterloo	Guelph	500	June 1993	A	Open	—	Yes
	York	Guelph	2,000	June 1993	A	Open	—	Yes
	Wellington	Guelph	400	July 1993	A	Cage	—	Yes
	Waterloo	Guelph	2,500	July 1993	A	Cage	—	Yes
	York	Guelph	200	July 1993	A	Cage	—	Yes
	Wellington	Guelph	100	July 1993	A	Cage	—	Yes
	Waterloo	Guelph	500	Aug. 1993	A	Cage	—	Yes
	Waterloo	Guelph	400	Aug. 1993	A	Cage	—	Yes
	Wellington	Guelph	100	Aug. 1993	A	Cage	—	Yes
	Wellington	Guelph	200	Aug. 1993	A	Cage	—	Yes
	Halton	Guelph	200	Aug. 1993	A	Cage	—	Yes
	Brant	Guelph	400	Sept. 1993	A	Cage	—	Yes
	York	Guelph	400	Sept. 1993	A	Cage	—	Yes
	Halton	Guelph	200	Sept. 1993	A	Cage	—	Yes

[a] Mix Meggerdorf/Gelnhausen/Laboratory culture.

Table 2. Location, origin, method, and number of stages of *H. transversovittatus* released into North America and their fate regarding establishment

State/ Province	County	Releases					Established in	
		Origin	No.	Date	Stages	Method	1993	1994
Pennsylvania	Philadelphia	IIBC	120	Aug. 1991	A	Field cage	Yes	NA
	Philadelphia	IIBC	3,850	Aug. 1991	E	Field cage	Yes	NA
	Philadelphia	IIBC	250	July 1992	A	Open	Yes	NA
New York	Genese	IIBC	396	Aug. 1991	A	Field cage	Yes	NA
	Genese	IIBC	11,350	Aug. 1991	E	Field cage	Yes	NA
	Genese	IIBC	200	July 1992	A	Open	Yes	Yes
	Genese	IIBC	200	July 1992	A	Field cage	Yes	Yes
	Genese	IIBC	4,300	July 1992	E	Field potted plants	Yes	Yes
Maryland	Prince Georges	BARC	3,605	Aug. 1992	E	Open	—	?
	Howard	BARC	315	Sept. 1993	E	Open	—	Yes
Virginia	Wise	IIBC	53	July 1992	A	Open	—	?
	Wise	quarantine	150	Aug. 1992	E	Open	—	?
Minnesota	Ramsey	IIBC	2,520	Aug. 1992	E	Laboratory potted plants	—	—
Washington	Whitman	IIBC	1,500	Aug. 1992	E	Field cage	No	—
	Whitman	IIBC	48	June 1993	A	Laboratory colony	—	—
	Whitman	WA	439	June–Aug. 1993	E	Field cage	Yes	NA
	Whitman	IIBC	12	June 1993	A	Field cage	—	?
	Grant	WA	471	July–Aug. 1993	E	Open	Yes	Yes
Oregon	Marion	IIBC	1,200	Aug. 1992	E	Field potted plants	Yes	Yes
	Marion	OR	20	Aug. 1993	A	Open	—	Yes
	Polk	OR	24	Aug. 1993	A	Open	—	Yes
	Polk	WA	300	Aug. 1993	E	Open	—	Yes
Montana	Gallatin	IIBC	250	Aug. 1992	E	Laboratory potted plants	—	?
Colorado	Denver	NY	100	Aug. 1993	E	Field potted plants	—	Yes
	Palisade	OR	8	Aug. 1993	A	Laboratory colony	—	—
Ontario	Wellington	IIBC	480	Aug. 1992	E	Field potted plants	Yes	Yes
Manitoba	Holland	Lethbridge	40	Oct. 1992	L	Field potted plants	Yes	Yes

NA, not analyzed.

amount of litter or the number of dead above-ground shoots is important for overwintering survival. In each of the 8 cages at the Pennsylvania site, purple loosestrife density was estimated by counting the number of live shoots in a randomly selected quarter of the cage in early September. Dead above-ground stems in 2 randomly selected 0.5-m^2 quadrats were harvested from each of the 8 cages. All litter in these quadrats was removed to bare soil. Stems and litter were kept separate in Berlese funnels in the laboratory for 1 wk at room temperature. Emerging adult *Galerucella* were collected daily. After 1 wk, all material was dried at 37°C for 3 wk and weighed.

Table 3. Preplanned contrasts comparing treatments at New York site for the survival of *Galerucella* spp.

Contrast	Mean	SEM	Mean square	$P > F^a$
1. Southern population vs	0.06	0.013	0.0078119	0.420
northern population	0.09	0.013		
2. Small cage vs	0.06	0.014	0.0002799	0.878
large cage	0.06	0.013		
3. 200 beetles released vs	0.08	0.013	0.0639292	0.027
600 beetles released	0.18	0.023		
4. 20 beetles released vs	0.11	0.055	0.0052792	0.507
60 beetles released vs	0.04	0.014		
180 beetles released	0.06	0.017		

[a] ANOVA was performed on arcsine square-root transformed data as proportion of individuals surviving. Means and SEM are given as untransformed proportions.

Data Collection and Statistical Analysis. In New York, successfully overwintered adult *Galerucella* were hand-collected and removed from the cages 3 times during the period from mid-May to early June 1993. Adults were counted and species composition determined. Counts of *Galerucella* spp. in Pennsylvania cages were made mid-June and early September 1993, but individuals were not removed. The proportion of successfully overwintered (established) insects in each cage was calculated as the number of insects recovered divided by the number released. Proportions were subjected to an arcsine square-root transformation (Draper and Smith 1981) before analysis with ANOVA (PROC GLM, SAS version 6.08) (SAS Institute 1990). Differences between treatment means, with regard to the New York data, were tested with 4 preplanned single degree of freedom contrasts (Table 3). Contrast 1 compared overwintering survival of beetles from southern Germany (Gelnhausen) (n = 6 cages) versus those from northern Germany (Meggerdorf) (n = 11). The 2nd contrast compared survival of Gelnhausen beetles in small cages (n = 4, 180 beetles) against large cages in which similar numbers of beetles had been released (n = 6, 200 beetles). The remaining 2 contrasts evaluated survival from different release numbers of *Galerucella*; 200 (n = 11) versus 600 (n = 4) Meggerdorf beetles in large cages, and 20 (n = 4) versus 60 (n = 4) versus 180 (n = 4) Gelnhausen beetles in small cages. Sum-

mary statistics are presented for untransformed data.

Evaluation of the 3 litter factors studied in Pennsylvania were each analyzed with 1-way analysis of variance (ANOVA). Differences between the 4 cages that had successfully established beetles were compared with the 4 cages in which the beetles failed to establish.

Results

Collection and Shipment. In 1992, a total of 35,000 *Galerucella* spp. adults was sent to North America. Mortality during shipment from Europe was low, only 457 individuals died, but mortality increased with increased handling time. Approximately 3,000 (10%) died in quarantine during the week following shipment. From quarantine, beetles were shipped overnight to cooperators in various states and Canada. The percentage of mortality was <10% if beetles were released shortly after receipt, but survival rates dropped to 50% if insects were kept in the laboratory an additional week.

Adult *H. transversovittatus* are very robust and mortality was low throughout all phases of handling. The percentage of mortality during shipment from Europe until field release was ≈2%. In 1992, an estimated 13,000 eggs were sent in moist florist foam, and 10,950 were released in North America by cooperators into potted plants or into plants in the field (see Blossey [1993] for rearing details). Establishment rates of larvae were ≈1%. The high percentage of mortality was attributed to inexperienced personnel extracting and inoculating eggs.

Releases. Establishment of both *Galerucella* species was confirmed at all 1992 release sites regardless of initial numbers, open field, or caged releases (Table 1). Adults successfully overwintered and produced a new generation. Insects became active according to local host-plant phenology, indicating their adaptation to various climates across North America. Insects even established in low numbers at sites in Pennsylvania, Minnesota, and Oregon that were flooded in spring or summer. The overall survival rate of *Galerucella* spp. at all 1992 release sites was estimated at 10%. Establishment of beetles released in 1993 were evaluated in spring 1994.

Establishment of *H. transversovittatus* was confirmed in spring 1993 at all sites checked for the presence of larvae (New York, Pennsylvania, Oregon, and Manitoba). Because of the limited sampling scheme, no information on the abundance of *H. transversovittatus* is yet available. A new generation of beetles emerged from laboratory colonies in Ontario, Alberta, New York, Maryland, Minnesota, and Oregon, some of which were field released in 1993 (Table 2).

Experiments. *Climatic Matching.* The different climatic preadaptation of the 2 source populations for *G. calmariensis* and *G. pusilla* did not influence their establishment at the New York site (contrast 1, Table 3). The survival of beetles from Gelnhausen (6.1%) and Meggerdorf (8.6%) was not significantly different ($P = 0.420$). Survival at the Pennsylvania site was also not significantly different between source populations ($P = 0.495$). However, continuous flooding from March through July considerably reduced overwintering success in Pennsylvania. Beetles survived in only 4 of the 8 cages.

Number Released. Survival was not significantly different in cages with 20, 60, or 180 beetles released (contrast 4, Table 3). However, survival of beetles in cages receiving 600 beetles was significantly higher than in cages with 200 beetles (contrast 3, Table 3).

Confinement. Survival in small and large cages was almost identical (6.0 and 6.1%, respectively), and differences were not significant (contrast 2, Table 3). The proportion of each species in the cages remained unaltered. *G. calmariensis* and *G. pusilla* from Meggerdorf and Gelnhausen had identical survival.

In open-field releases, a proportion of the released beetles immediately dispersed, often in what appeared to be long-distance flights. Individuals flew several meters straight up from the vegetation canopy, and, because of their small size, were out of sight after a few meters. The distance traveled remains unknown. However, overwintered adults and a new generation of larvae were observed in unconfined releases at both United States and Canadian release sites. Population estimates were difficult because beetles dispersed and quantitative sampling was avoided to allow population buildup.

Litter Influence. Beetles successfully overwintered in 4 of the 8 cages at the Pennsylvania site (Table 4). Two of the cages had received beetles from Meggerdorf and 2 from Gelnhausen. Beetles survived continuous flooding from March through July and produced a new generation in all 4 cages. A few adults and eggs from the open field release were also found outside the cages. The differences in survival among the cages with and without successful establishment of *Galerucella* spp. was not attributable to differences in the number of live stems ($P = 0.385$), dry stem weight ($P = 0.806$), or dry litter weight ($P = 0.222$). The number of adults collected in the laboratory indicated that most beetles were preparing to overwinter in the litter. Earlier observations of a few beetles remaining in the dry stems were confirmed (Table 4).

Discussion

All 3 species have reproduced successfully in the field and have passed the most critical phase for establishment in North America. An analysis of past weed biological control attempts has shown that of those agents that failed to become established, ≈80% never completed a single generation

Table 4. Survival and reproduction of *Galerucella* spp. relative to number of living stems, leaf litter, and standing dead stems in each cage at the Pennsylvania site, 1993

Population source	Overwintered adults per cage (June 1993)	F_1 adults in two 0.5-m^2 quadrats (Sept. 1993)		No. live stems per cage	Avg dry wt litter per 0.5 m^2, g	Avg dry wt stems per 0.5 m^2, g
		In litter	In stems			
Southern	8	22	3	149	137.5	162.5
Southern	94	48	2	134	44.5	156.5
Northern	20	1	0	129	41.5	124.0
Northern	1	0	0	91	46.0	63.0
Southern	0	0	0	117	111.5	133.5
Southern	0	0	0	72	166.5	173.0
Northern	0	0	0	127	151.5	110.0
Northern	0	0	0	121	40.0	117.0

in the country of introduction (Crawley 1986). Extinction of control agents after completing 1 or more generations accounts for only ≈20% of failures (Crawley 1986).

Mortality of adult chrysomelids during shipping and handling within North America indicated that adults were stressed when they were released in 1992. Releases occurred at a time when newly emerged adults normally prepare for overwintering and disappear from the host plants. Reduced activity levels were obvious in the rearing cages and beetles aggregated in the litter.

Collection of larvae and shipment of newly emerged adults from Europe was necessary to avoid the introduction of adult or larval parasitoids. Recommendations for redistribution (Malecki et al. 1993) now include shipment of beetles during their early ovipositional period and an initial release in cages to avoid rapid dispersal. This ensures that an F_1 generation will be produced at the release site, allowing the new generation of beetles to prepare for overwintering according to local climates. The population size of both *Galerucella* spp. in initial 1992 releases dropped to ≈10% during the winter. Experiments with beetles kept outdoors in cages in northern Germany showed a survival rate of ≈30% (Blossey 1991). Differences are most likely the result of the stress beetles experienced during handling and shipment. We expect the survival rate of beetles in the field in future years to be much higher.

Different climatic preadaptations of the 2 *Galerucella* species did not influence the initial establishment of control agents. Climatic differences between the 2 source populations may have not been sufficient to cause significant effects. The general distribution patterns of the 2 *Galerucella* species in Europe suggests that *G. pusilla,* which is less abundant in Scandinavia (Palmén 1945), should be less successful in the northern part of the *L. salicaria* distribution in North America. Time is needed to evaluate whether both species will spread and be effective throughout the entire North American distribution of *L. salicaria.* Until now, no differences in areas colonized by either species of *Galerucella* have been documented.

Gallerucella calmariensis and *G. pusilla* successfully established in a wide range of climatic conditions and adapted to local plant phenology. Adults most likely responded to changes in temperature rather than photoperiod for their reappearance in spring. This tight link to the host-plant phenology will aid in their establishment across the range of habitats currently occupied by *L. salicaria* in North America.

Stochastic events, such as adverse weather, might still eradicate some newly established populations. Two populations in Minnesota and Ontario appeared to become extinct after initial establishment. They were affected by spring floods (Ontario) or by the June–July floods of the Mississippi river (Minnesota) in 1993. In Minnesota, virtually all of the loosestrife at the release site was totally submerged for several weeks, and beetles were 3rd–4rth instars at the onset of the flood. However, individuals survived the flood, and adults and eggs were observed on a number of plants on 26 May 1994 (D. Andow, University of Minnesota, St. Paul, and L. Skinner, Minnesota Natural Resources, personal observation). Flooding was suspected as a reason why one 1993 Vancouver release of *G. calmariensis* and the 1992 New Brunswick release of *G. pusilla* failed to establish. Also, continued flooding at a release site in Oregon and Pennsylvania did not completely prevent establishment, but survival was low. Because beetles in Oregon were open-field released, adults might have moved to higher ground, thus avoiding floodwaters. In Pennsylvania, cages were flooded (up to 80 cm of standing water) from March through early July. The ability to survive in such a situation, in which no escape to higher ground was possible is remarkable. Some adults might have overwintered in the dry inflorescences of standing dead stems or moved up old plants during warm spells. Activity of adults on warm days in February has been observed in earlier overwintering experiments (B. B., unpublished data). Further studies are needed to confirm methods of survival under flood conditions.

Our results show that survival rates of *G. calmariensis* and *G. pusilla* improve with the size of

the released population. The reason for the higher survival rates in cages with 600 compared with 20, 60, or 200 initially released adults remains unclear. Possible explanations are (1) local predator saturation or (2) increased survival of beetles in larger aggregations. Predator saturation seems unlikely because from November through April, cages were left in the field without screening and predators could freely move among the cages. Cages with higher numbers of released beetles would have suffered more from predator exploitation if predators concentrated in patches with higher prey densities. But survival of beetles in cages did not support this concept. Moreover, large spring populations of the fourlined plant bug, *Poecilocapsus lineatus* (F.) (Heteroptera: Miridae), a common herbivore of *L. salicaria* (Hight 1990), built up inside field cages, whereas damage was inconspicuous outside the cages. This indicated that natural enemies were effectively excluded by the cage screening.

Aggregation of adult *Galerucella* spp. may have an effect on survival either during the breeding season or during overwintering. Aggregation in shelters during overwintering, generally interpreted as a result of a limited supply of suitable microhabitats (Danks 1987), is known for various taxa, including beetles. Limited supply of suitable microsites can be excluded in this study because survival in small and large cages was identical. Both *Galerucella* species aggregate during their ovipositional period, and aggregations were observed in early spring at the base of sprouting plants (Blossey 1991). Whether individuals actually favor large aggregations, and whether survival is affected by the size of aggregations, need to be determined experimentally. It also remains unclear why large aggregations should be less vulnerable, because 1 of the main mortality factors in the field is the entomophagous fungus *Beauvaria bassiana* (Balsamo) Vuillemin (B. B., unpublished data). The spread of this fungus seems to be favored by beetles in aggregations.

The ability of beetles to survive in 4 of the 8 cages at the Pennsylvania site despite continuous flooding could not be attributed to any of the measured parameters. The number of standing dead stems or amount of litter was not significantly different between cages with and without establishment. Because the release site consisted of a monoculture of purple loosestrife, differences in the perceived quality of the litter seem unlikely. Most likely, the number of individuals resting or overwintering above the floodwater level determined whether or not beetles survived the winter.

Survival of released *Galerucella* spp. was generally good in western Canada (Table 1); however, failure to establish for some releases of *G. calmariensis* may be attributed to infection of adults with the native fungal disease, *B. bassiana*. Early laboratory colonies of *G. calmariensis* at Lethbridge were reared from egg to adult on potted purple loosestrife. *B. bassiana* spores were present on the soil surface, and infection of larval and adult *G. calmariensis* was common and difficult to control. Containment and reduction of the disease in the laboratory was eventually attained by hatching eggs in sterile petri dishes and rearing small batches of larvae on cut plant material in sealed plastic boxes. Although care was taken to ship healthy insects for release, dead adult *G. calmariensis* infected with fungus were noted in the field after some of the initial releases made in Manitoba (C. Lindgren, Manitoba Purple Loosestrife Project Coordinator, personal correspondence).

Most Canadian releases of the 2 leaf beetles consisted of laboratory-reared adults whereas the U.S. releases (except 1) were from field or outdoor rearings. It will be interesting to compare the establishment success of these 2 rearing methods. Laboratory cultures were kept at long days (photoperiod of 16:8 [L:D] h) and constant temperatures (25°C). Observations in New York indicated that released beetles coming from a longer photoperiod than experienced in the field went into overwintering. After a short feeding period, they disappeared from the host plants. Dispersal did not account for this phenomenon because it was also observed in cages. It will be important to see whether beetles will survive almost a year without feeding. Most likely, the photoperiod of the cultures in the rearings will have to be changed to mimic more accurately conditions found for beetles once they are released. Harris (1984) noted a similar phenomenon with spring releases of a laboratory-reared beetle, *Rhinocyllus conicus* (F.), that went into diapause immediately after release, but survived until the next spring.

Obtaining accurate estimates on the density of established *H. transversovittatus* will require additional study. At those locations at which roots were checked for feeding damage, the presence of weevils was confirmed. The rearing initiated across North America should provide larger numbers of *H. transversovittatus* in future years for further releases and redistribution. Despite the slow generational turnover, we are confident that the destruction caused by larval feeding will be very important in controlling purple loosestrife in North America (Malecki et al. 1993).

USDA–ARS terminated its involvement in the purple loosestrife biological control program on 1 October 1993. Justification for termination included funding constraints, the perception that the plant was of little importance to U.S. agriculture, and that end-user groups had not raised their concerns about this weed to ARS administrators. However, biological control of purple loosestrife continues under the direction of the National Biological Survey, U.S. Department of Interior (Malecki et al. 1993).

The 1st goal, establishing populations of control agents against purple loosestrife in North America, has been achieved. Three introduced species have

reproduced successfully in the field and are past the most critical stage. Current efforts are focused on population propagation and redistribution programs to make insects available for further releases. At the same time, we are developing methodologies for monitoring established insects and their damage to purple loosestrife. Throughout these phases, basic research will accompany releases to answer questions about the effect of single or multispecies herbivory on plant performance, the extent of control achieved by different agents, and the spread of agents. These data will not only guide future efforts in the control program against purple loosestrife but also improve the success rate and predictability of future efforts aimed at controlling invasive weeds with biological control.

Acknowledgments

We acknowledge J. Drea, R. Malecki, and D. Schroeder for continual support, guidance, and involvement in this project. The European research and rearing was carried out by the International Institute of Biological Control, European Station, Switzerland at facilities of the Christian-Albrechts-University, Kiel, northern Germany. G. Petersen, R. Lietz, A. Blossey, T. Grünkorn, D. Babendreier, R. Nötzold, J. H. Steffen, and G. Lebs assisted in insect collections and rearings in Europe. L. Kok, L. Skinner, D. Sreenivasam, D. Andow, I. Savov, T. Leahy, G. Piper, N. Rees, E. Coombs, L. Johnson, P. McEvoy, M. Huso, R. Cranston, C. Lindgren, K. McCully, J. Myers, D. Ralph, and J. Stewart, together with numerous volunteers, released and monitored insects in various states and provinces, and provided information incorporated in this article. The field assistance of D. Carrol, J. Hickey, and K. Kos in New York, of S. Hanna and S. Watson in Pennsylvania, G. Bowles and C. Medina in Ontario, and S. Verzosa and E. Pavlik in Lethbridge is acknowledged. J. Adams checked beetles for presence of pathogens, and G. Duke is acknowledged for the culturing and identification of *B. bassiana.* Final preparation of the manuscript was supported in part by a grant from the Maryland Agricultural Experiment Station to S. Hight.

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Received for publication 26 August 1994; accepted 6 February 1995.