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Population Dynamics and Long-term Effects of *Galerucella* spp. on Purple loosestrife, *Lythrum salicaria,* and non-target native plant communities in Minnesota.

BY

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Abstract
A field study was conducted to assess population dynamics and long-term effects of the biological control agent *Galerucella* spp. on Purple loosestrife, *Lythrum salicaria,* and non-target native plant communities in Minnesota. Five *Galerucella* spp. release sites in central and southern Minnesota were studied between 1995 and 2003. *Galerucella* spp. established at all five release sites following additional release of insects at three locations. At all five release locations, *Galerucella* spp. populations peaked between three and five years after successfully establishing. As a result, purple loosestrife densities, height and flowering were reduced across all sites. After the initial peak in *Galerucella* spp. densities, all sites saw a decline of *Galerucella* spp. abundance in response to the reduction in purple loosestrife abundance. *Galerucella* spp. and loosestrife abundance followed two distinct patterns over time. The *Galerucella* spp. populations either rebounded with increasing loosestrife abundance or the *Galerucella* spp. population did not rebound. Our results suggest that *Galerucella* spp. can provide effective control of purple loosestrife and increase plant species richness. However, there may be limitations whereby some insect populations decline precipitously after reaching high densities and do not recover following declines or have not been observed to recover in the time frame of this study. Continued monitoring will be needed on those sites thatn did not rebound to determine if the *Galerucella* spp. populations will once again increase and control the purple loosestrife without reintroducing the beetles.

1. Introduction

One of the major criticisms of weed biological control of weeds is the lack of post-release studies that document the long-term effects of the introduced agents (Blossey and Skinner 2000, McClay 1995, McEvoy and Coombs 1999). Most post-release monitoring efforts have focused on agent establishment and spread with little quantitative data on host suppression (Crawley 1989, McClay 1995). In particular, there is a need to document control agent populations over time and effects on the target pest plant and associated plant communities. Such studies can provide knowledge of success or failure of a biological control effort but also provide insight to predict outcomes of future biological control programs better (Blossey and Skinner 2000, McFayden 1998, McEvoy and Coombs 1999). Classical biological control of purple loosestrife, *Lythrum salicaria* L., in North America provides an opportunity to develop long-term studies on the impact of release biological control agents.

Purple loosestrife, *Lythrum salicaria* L., is a perennial emergent wetland plant introduced into North America from Europe (Stuckey 1980, Thompson et al. 1987). Since its introduction, purple loosestrife has become established across the northern half of the United States and Canada (Stuckey 1980). Purple loosestrife is a herbaceous perennial which forms a woody crown from which new shoots emerge every year (Shamsi and Whitehead 1973). Seed dispersal, rather than vegetative reproduction is the major means of dissemination. It is estimated that each plant is capable of producing up to 2.7 million seeds per season (Thompson et al. 1987). The prolific seed production and subsequent seed rain leads to the creation of an extensive seed bank (Welling and Becker 1990). Once a seedbank is established, purple loosestrife more successfully colonizes disturbed and open sites than do native species (Thompson et al, 1987, Welling and Becker 1993).

Invasions by purple loosestrife have been associated with ecosystem impacts including reduction of native plant diversity and abundance, reduction in wildlife habitat, and changes to wetland function as described by Blossey et al. (2001a). In particular, there are numerous studies where purple loosestrife has been shown to be highly competitive compared with other native wetland species (Gaudet and Keddy 1988, Gaudet and Keddy 1995, Mal et al. 1997, Rawinski and Malecki 1984, Weiher et al. 1996, Weihe and Neely 1997, Welling and Becker 1990).

Efforts to manage purple loosestrife with conventional control methods such as chemical application, cultural practices and mechanical removal, provide only limited, short-term control and are only effective on small populations (Blossey et al. 2001b, Skinner et al. 1994, Welling and Becker 1993, Welling and Becker 1990). Experience in Minnesota suggests that controlling large, established populations of purple loosestrife with conventional methods is rarely successful because of the large seedbank allows the population to rebound following control (Skinner et al. 1994, Welling 1990).

Classical biological control is considered an alternative to conventional control methods and may provide long-term control of purple loosestrife (Blossey et al. 1996, Malecki et al. 1993). In 1992, *Galerucella calmariensis* L. and *G. pusilla* Duft. (Coleoptera: Chrysomelidae) were introduced to control purple loosestrife in North America and have become established across the north temperate portion of the United States and Canada (Hight et al. 1995, Lindgren et al. 2002). Since 1992, there have been a number of reports documenting the establishment, control success and non-target impacts caused by *Galerucella* spp. (Blossey 1995, Blossey et al. 2001a, Blossey et al. 2001b, Blossey and Skinner 2000, Corrigan et al. 1998, Dech and Nosko

2001, Katovich et al. 1999, Katovich et al. 2001, Kaufman and Landis 2000, Landis et al. 2003, Lindgren 2000, Lindgren 2003).

In Minnesota, *Galerucella* spp. were first released for the biological control of purple loosestrife in 1992. Since then, more than eight million beetles have been released on more than 800 purple loosestrife infestations statewide. To effectively evaluate the biological control program within Minnesota, long-term monitoring was initiated. The objectives of our studies were to quantitatively assess the population dynamics of *Galerucella* spp. as well as document their impacts on purple loosestrife and associated wetland plant species for up to nine years post-release at multiple sites.

2. Materials and Methods

2.1. Study sites and *Galerucella* spp. releases

Five study sites were chosen in central and southern Minnesota based primarily on their histories of having the earliest releases of *Galerucella* spp. in the state. The sites are located near the following cities or lakes: Winona, Reno, Circle Lake, White Bear Lake and Big Marine Lake.

Winona, MN. The Winona site is a 3.2 ha palustrine wetland located in southeastern Minnesota near the Mississippi river in Winona County and within the city limits of Winona (Table1). Although the wetland is near the Mississippi river, the wetland is recharged by overland flow from nearby blufflands and runoff from impervious surfaces (roads and parking lots). For much of the year, a portion of the Winona wetland has standing water, while the edges tend to have saturated soils. The Winona wetland vegetation community had been dominated by purple loosestrife for more than 20 years and at the time of release was essentially a monoculture of purple loosestrife covering 95% of the wetland with only a few native plants found around the margins of the wetland. The plant community other than loosestrife consisted of cattails, *Typha* spp., rushes, *Scirpus* spp., a variety of sedges, *Carex* spp., and grasses, *Gramniae* spp. *Galerucella calmariensis* and *G. pusilla* were first introduced in 1993 when 1,000 adults were released directly on to loosestrife plants. The insects released were collected immediately prior to their release from Germany where they were field collected and shipped to Minnesota. The insects were a mixture of the two species, however, there was no determination of the percentage of each. Visual surveys carried out in 1994 and 1995 found little evidence of *Galerucella* spp. establishment with only a few adults and egg masses found each year. Consequently, more than 4,000 *Galerucella* spp. were released in 1995 and 6750 in 1996, in an effort to establish the control agents in this wetland (Table 1). The 1995 and 1996 releases were made from colonies reared on loosestrife plants in cages out doors and in the greenhouse at the University of Minnesota as described by Loos and Ragsdale (1998).

Reno, MN. The Reno site is an 11 hectare palustrine wetland located in Houston County near the border with Iowa and Wisconsin about 2.5 miles south of the city of Reno, MN (Table1) This wetland is a backwater area of the Mississippi river and is prone to seasonal flooding. The Reno site had also been dominated by loosestrife for more than two decades. Associated plant species were cattail, bur-reed, *Sparganium eurycarpum*, and bulrush, *Scirpus validus*. One thousand adult *Galerucella* spp. were released directly on to purple loosestrife plants in 1993. As in the Winona site, the insects were part of the same collection from Germany. The *Galerucella* spp. failed to establish two years after release, therefore an additional 4000 *Galerucella* spp. were released at the same location in 1995 from adults reared on plants in a greenhouse during late winter and early spring. The subsequent releases were made by adult

beetles were released by placing fine meshed sleeve cages over purple loosestrife plants and then placing 200 to 300 beetles within each cage (referred to as the sleeve cage method). The sleeve cages were removed one week later after egg deposition had occurred.

White Bear Lake, MN. The White Bear Lake location is a 13.8 hectare wetland in Ramsey County in east-central Minnesota within the city limits of White Bear Lake (Table 1). This is a shallow wetland that is seasonally flooded and largely dominated by cattail, except for the southern one third of the wetland, which is dominated by purple loosestrife. In 1993, this site received its first release of 1,000 adult *Galerucella* spp. from same collection and shipment from Germany as the Winona and Reno sites. Similar to the Winona and Reno sites, only a few egg masses and adult *Galerucella* spp. were observed in 1994 and no evidence of beetles were observed in 1995. Consequently, more than 4,000 laboratory and greenhouse reared *Galerucella* spp. were released in 1995 using the sleeve cage method.

Circle Lake, MN. The Circle Lake site is a 25 ha palustrine wetland located along the lakeshore of Circle Lake in Rice county (Table 1). This shallow marsh is semi-permanently flooded with a gradient from saturated soils to standing water. The wetland is approximately 50-200 meters wide ringing two thirds of the lake edge. The vegetation was 50% dominated by purple loosestrife with a diversity of native plants such as sedges, *Carex* spp., river bulrush, *Scirpus fluviatilis,* and smartweeds, *Polygonum* spp., at the drier edge and cattail and bur-reed at the wet edge adjacent to the lake itself. The loosestrife had been established at the site for over 20 years and had spread throughout the wetland complex. 500 greenhouse reared *Galerucella* spp. were released in 1994 using the sleeve cage method.

Big Marine Lake, MN. The Big Marine Lake site is a 26 ha palustrine emergent shoreline located in Washington County in east-central Minnesota. This site is a wet meadow that has saturated soils and predominant vegetation type is sedges and grasses. Purple loosestrife was found throughout the wet meadow with areas where purple loosestrife was the dominant plant. This site, however, is not considered to have a monoculture of purple loosestrife. Adjacent to this wet meadow is another large wet meadow 40 hectares in size that was dominated by purple loosestrife. The first release at this location was in 1998. The *Galerucella* spp. for their release were captured earlier in the year from the Circle Lake site and placed on potted plants inside a sleeve cage. The *Galerucella* spp. reproduced within the cage and approximately 7,000 F_1 offspring were released by placing potted purple loosestrife plants with larvae, pupae, and new emerged adults next to purple loosestrife plants at this site. An additional estimated 21,000 beetles were released in 1999 using the same release technique.

2.2. Sampling design

To monitor changes in insect and plant communities over time within each site, we adapted the standardized monitoring protocol described by Blossey and Skinner (2000). Transects, 50m to 75m in length, were established at each field site. Permanent $1m^2$ quadrats were placed every 12.5 meters along each transect. The corners of each quadrat were marked with posts. Six transects with a total of 30 quadrats were established at the Circle Lake site in 1995 (Table 2). Four transects were place near the original release point, while two transect were placed 400m away to serve as controls. In 1997, four additional sites including Big Marine Lake, Reno, White Bear Lake and Winona, were established with two transects each at least 50m apart near the initial release point. Five to seven permanent quadrats were established on each transect for a total of 11to 14 quadrats at each site (Table 2).

2.3. Sampling *Galerucella* spp., purple loosestrife and other vegetation

At each location, the quadrats were non-destructively sampled twice each year. Sampling occurred once in the spring to capture *Galerucella* spp. abundance and once in late summer to capture impacts to purple loosestrife and abundance of other plant species present. In the spring (late-May to early-June), we timed our sampling to coincide with the phenology of purple loosestrife plants. Sites were sampled when the majority of the loosestrife plants ranged from one to three feet in height. Due to a faster accumulation of growing degree-days at southern latitudes, sites were surveyed from south to north over a three week period. This was to ensure *Galerucella* spp. presence and oviposition was occurring and could be quantitated at each site. At each location, each quadrat was sampled for the number of *Galerucella* spp. egg masses, larvae and adults. This was carried out by visually counting each insect life stage separately. We counted the adults first as they were likely to drop off the plants if disturbed, and then counted the number of larvae and egg masses. In 2004, 200 *Galerucella* spp. were collected from four of the five sites for species identification. The first 100 male beetles were dissected and identified using morphological characteristics of the adeagus to provide a ratio of each species present.

Quadrats were revisited in late summer (late August) to record purple loosestrife percent cover, number of stems, height (five tallest plants) and the total number of inflorescences. In addition, the percent cover for each species present other than purple loosestrife was visually estimated. Sites were revisited each year for up to 9 years after release.

2.4. Data Analysis

Due to the variability of the sites and insect releases, we chose to analyze each site separately and standardize for number of years after *Galerucella* spp. introduction. Each quadrat was treated as a replicate in a completely randomized design. The number of quadrats (replicates) for each site is found in Table 2. For each site, the mean ± SE of *Galerucella* spp. egg mass density, purple loosestrife stem density, percent visual cover, total number of inflorescence, stem height and number of plant species other than purple loosestrife, were calculated for the number of years after initial release. We chose to use egg mass density as our indicator of *Galerucella* spp. abundance because the adults tend to aggregate and move readily with in a site and larvae can be hidden in the apical meristems of the plant. Egg masses are easily observed; they are stationary and remain on the plants for up to two weeks, providing a manageable timeframe in which to conduct the surveys. Analysis of variance (ANOVA) and Ryan-Einot-Gabriel-Welsch Multiple Range Test (PROC GLM, SAS Institute 2001) were used to analyze differences among the number of years after release for density of *Galerucella* spp., purple loosestrife variables, and number of species other than purple loosestrife observed.

3. Results

Galerucella spp. established at all five release sites following additional release of insects at three locations. *Galerucella* spp. did not establish after the initial release of adults in late summer at Reno, White Bear Lake and Winona. The initial releases at these three sites were from *Galerucella* spp. collected in Europe and shipped to Minnesota in July of 1993. After two years of finding very little evidence of establishment at Reno and Winona, and no evidence of *Galerucella* spp. at White Bear Lake, additional releases were made with adults reared in

outdoor cages on potted plants, with the potted plants containing primarily pupae and adults of the F_1 generation.

Circle Lake was the first site in Minnesota where *Galerucella* spp. became established. This initial release in1994 was made using sleeve cages on purple loosestrife plants to confine the beetles, with the hope that mating and egg laying would occur before cages were removed and insects could disperse. One week after initial release, sleeve cages were removed and we observed mating pairs and high numbers of egg masses of *Galerucella* spp. on each plant. In subsequent observations during the year of release, we observed hundreds of larvae that eventually defoliated the purple loosestrife plants on which the beetles were initially placed. One year after release, *Galerucella* spp. were observed scattered up to 100 meters from the original release point. G*alerucella* spp. egg mass densities fluctuated significantly ($F= 7.38$, df= 6,197; $P= <0.0001$) over time. Mean number of egg masses per m^2 ranged from a high of 22.7±4.6 four years after release to a low of 1.1±0.3 nine years after release (Figure 1a). As the *Galerucella* spp. densities peaked, the first impacts were a reduction in purple loosestrife height and number of inflorescences. (Figure 1c-d). This was followed by reduction in stem densities (Figure 1b). *Galerucella* spp. populations cycled from high to low densities over the 9-year period with a second peak density measured seven years after the initial release. Even with the population fluctuations, purple loosestrife stem density, height and flowering did not rebound (Figure 1b-d). In particular, stem density steadily declined and total number of inflorescence remained near zero for the past six years. The number of species other than purple loosestrife changed over time ($F= 6.52$, df= 6, 202; $P= <0.0001$). Six years after release the number of species other than purple loosestrife peaked at 3.8 ± 0.3 species per m^2 compared to low of 2.2 ± 0.2 three years after release (Figure 1e). Outside the study plots, we observed *Galerucella* spp. up to 1.5 km from the release point four years following the initial release.

In Winona, *Galerucella* spp. became established and egg mass densities remained above 20 egg mass per m^2 for the first three years after additional releases were made in 1995 and 1996 (Figure 2a). A subsequent reduction in purple loosestrife flowering, and stem height, followed by a reduction in stem density occurred by three years after release (Figure 2b-c). As the purple loosestrife stem density was reduced to near zero four years after release, egg mass density declined sharply (Figure 2a-b). The lack of purple loosestrife continued to cause a decline in *Galerucella* spp. egg mass density five years after release. With a lack of insect pressure, the purple loosestrife rebounded in stem density and stem height, while flowering continued to be suppressed. *Galerucella* spp. responded to the purple loosestrife increase with a spike in egg mass densities (Figure 2a), which in turn, was followed by a reduction in purple loosestrife stem density and height (Figure 2b-c). Over the seven-year period, the number of plant species other than purple loosestrife increased ($F= 10.78$, df= 6,84; $P= <0.0001$). The number of species other than purple loosestrife increased from a low of 0.4±0.2 one year after release to a high of 2.4 ± 0.3 species per m^2 four years after release (Figure 2b,e).

Egg mass densities fluctuated dramatically at White Bear Lake ($F= 11.47$, df= 6, 70; $P= <0.0001$)(Figure 3a) and Reno ($F= 11.41$, df= 6, 73; $P= <0.0001$)(Figure 4a) over time. Both sites followed similar patterns with *Galerucella* spp. populations peaking five years after release then collapsing to near zero by eight years after release (Figures 3a and 4a). Egg masses per m^2 reached a peak of 80.5 ± 20.9 at White Bear Lake and 85.8 ± 22.4 at Reno, which were four times higher than the intial peak following release at Circle Lake or Winona. There was a corresponding decrease in purple loosestrife height ($F= 31.3$, df= 6, 69; $P= <0.0001$) and number of inflorescences ($F= 6.82$, df= 6, 69; $P= <0.0001$) at White Bear Lake (Figure 3c-d) and

decrease in purple loosestrife stem density (F= 3.25, df= 6, 73; *P*= <0.007), height ((F= 71.64, df= 6, 73; *P*= <0.0001), and number of inflorescences (F= 10.46, df= 6,73; *P*= <0.0001) at the Reno site (Figure 4b-d) corresponding and subsequent to increase in egg massess. The purple loosestrife rebounded, however, when *Galerucella* spp. declined at both sites (Figures 3a and 4a). Although there was some fluctuation of the number of plant species other than purple loosestrife at White Bear Lake (F= 6.68, df= 6,70; *P*= <0.0001), there was no difference between two and seven years after release (Figure 3e). There was no change in the number of plant species other than purple loosestrife at Reno (F= 2.23, df= 6,73; *P*= 0.05) over time (Figure 4e).

At Big Marine Lake, *Galerucella* spp. egg mass densities peaked four years after release (F= 13.38, df= 6,76; *P*= <0.0001) and declined sharply the following year (Figure 5). Following an initial increase in egg mass abundance three years after release, there was a marked reduction in purple loosestrife stems (F= 7.40, df= 6,76; *P*= <0.0001), height (F= 80.64, df= 6,76; *P*= <0.0001), and flowering (F= 26.76, df= 6,76; *P*= <0.0001)(Figure 5b-d). There was no change in the number of plant species other than purple loosestrife at Big Marine Lake (F= 1.93, df= 6,76; *P*= 0.087).

Dissections of *Galerucella* spp. collected from four of the five sites in 2004, suggest that two sites are dominated by *Galerucella calmariensis* (Circle Lake (90%) and Winona 94 %) and two sites are dominated by *G. pusilla* (Big Marine Lake (94%) and White Bear Lake (100%). No sample was obtained for the Reno location.

4. Discussion

At all five release locations, *Galerucella* spp. populations peaked between three and five years after successfully establishing. As a result, purple loosestrife densities, height and flowering were reduced across all sites, similar to the findings of Blossey and Skinner (2000), Landis et. al. (2003) and Lindgren (2003). Lindgren (2003) documented complete elimination of purple loosestrife stems, at one site, six years post release and a subsequent decline in *Galerucella calmariensis* abundance. In contrast, purple loosestrife remained present at all of our locations, albeit much reduced at three of the five locations. After the initial peak in *Galerucella* spp. densities, all sites saw a decline of *Galerucella* spp. abundance in response to the reduction in purple loosestrife abundance. After this initial peak, *Galerucella* spp. and loosestrife abundance followed two distinct patterns over time. The *Galerucella* spp. populations either rebounded with increasing loosestrife abundance or the Galerucella spp. population did not rebound.

At Winona and Circle Lake, *Galerucella* spp. populations rebounded (seven years post release) after egg mass densities neared zero. At Reno and White Bear Lake, *Galerucella* spp. populations have not rebounded since their initial declines six years post release and have remained low for two to three years. *Galerucella* spp. populations at all five sites suggest a density dependent relationship with purple loosestrife, but lack of a population rebound at Reno and White Bear Lake suggest that other factors may be influencing a population response. One such response may be stochastic effects that can occur with small insect populations that may cause small populations to go extinct locally. In particular, Allee affects and environmental variability play significant roles in insect establishment (Grevstad 1999a). Grevstad (1999a, 1999b) suggests that the combination of these two factors have an affect on establishment whereby establishment rate increases gradually with a concomitant increase in founder size.

Dominance by *Galerucella calmariensis* or *G. pusilla* may be reflected in beetle densities and control success at individual sites. At Circle Lake (nine years post release) and Winona

(seven years post release) where insect abundance and purple loosestrife control was sustained, the dominant species was *Galerucella calmariensis*. The dominant insect species at Big Marine Lake (five years post-release) and White Bear Lake (eight years post-release) where control was not sustained, was *Galerucella pusilla*. It is suspected that the dominant species released at all sites was *G. calmariensis*. In particular, the beetles introduced to Big Marine Lake were collected from Circle Lake, where the dominant insect is *G. calmariensis*. We speculate that the initial increase in *Galerucella* spp. abundance at most sites may be dominated by *G. calmarienis*, but at some sites, such as Big Marine Lake and White Bear Lake, the smaller remaining populations are predominantly *G. pusilla*. Further research is required as to if and why this may have occured.

Plant species richness increased at sites where purple loosestrife control was realized long-term, indicating long-term control of purple loosestrife is a key element in sustaining a diversity of native plant species. Treberg and Husband (1999) and Farnsworth and Ellis (2001) found no association between number of native wetland species and purple loosestrife. However, after disturbance events, such a broadleaf herbicide application (Gabor and Murkin 1996) or establishment of biocontrol agents (Landis et al. 2003) number and or density of native species increased. Gabor and Murkin (1996) reported an increase in number of grass seedlings after broadleaf herbicide treatments on purple loosestrife compared with control treatments. Our finding were similar to Landis et al. (2003), who reported an increase in native species richness as purple loosestrife plant height and percent cover declined after establishment of *Galerucella* spp.

Although our results were similar to Blossey and Skinner (2000) and Landis et al. (2003) at three to five years post-release, *Galerucella* spp. abundance and control success may vary over the long-term. Meta population dynamics may influence the re-colonization of sites, where *Galerucella* spp. population declines have occurred. At the Winona and Circle Lake locations, there are multiple wetlands, infested with purple loosestrife, that surround our study site. Three years post-release, *Galerucella* spp. had spread from the study site to other purple loosestrife infested wetland up to 15 km away. We speculate that after a *Galerucella* spp. population declines and the purple loosestrife rebounded, immigration of *Galerucella* spp. from nearby wetlands may aid in their re-establishment. Further study, however, is needed to confirm this hypothesis.

In conclusion, *Galerucella* spp. can provide effective control of purple loosestrife. However, there may be limitations whereby some insect populations decline precipitously after reaching high densities and do not recover following declines or have not been observed to recover in the time frame of this study. Continued monitoring will be needed to determine of the Galerucella spp. populations will once again increase and control the purple loosestrife without reintroducing the beetles. Control of purple loosestrife can increase species richness when control is sustained. We have observed distinct benefits of *Galerucella* spp. as a biological control agent.

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Table 1. Site characteristics and *Galerucella* spp. release information.

Site	County	Latitude	Longitude	Site Type	Cowardin	Species	Release Date(s)	Number Released
Big Marine Lake	Washington	45.20536 N	92.86505 W	Wet Meadow, saturated soils; lakseshore	PEM/SS1B	GC,GP	1998	7000
						GC,GP	1999	21000
Circle Lake	Rice	44.42256 N	93.36604 W	Shallow Marsh, semi-permanently flooded	PEMF	GC,GP	1994	500
Reno	Houston	43.59517 N	91.29186 W	Shallow Marsh, semi-permanently flooded	PEMFh	GC,GP	1993	1000
						GC,GP	1995	4165
White Bear Lake	Ramsey	45.09389 N	93.00183 W	Shallow Marsh, seasonally flooded	PEMCd	GC,GP	1993	1000
						GC	1995	3306
						GP	1995	937
Winona	Winona	44.03871 N	91.64974 W	Shallow Marsh, seasonlly flooded	PEMC	GC,GP	1993	1000
						GC	1995	2184
						GP	1995	2091
						GC,GP	1996	6750

GC= *Galerucella calmariensis*, GP= *Galerucella pusilla;* Cowardin refers to wetland classification system (Cowardin 1979)

Table 2. Sampling design information for five *Galerucella* spp. release sites in Minnesota.

Site	Number of Transects	Total Number of Quadrats	Number of Years Sampled
Big Marine Lake	2	12	7
Circle Lake	6	30	9
Reno	2	12	7
White Bear Lake	2	11	7
Winona	2	14	7

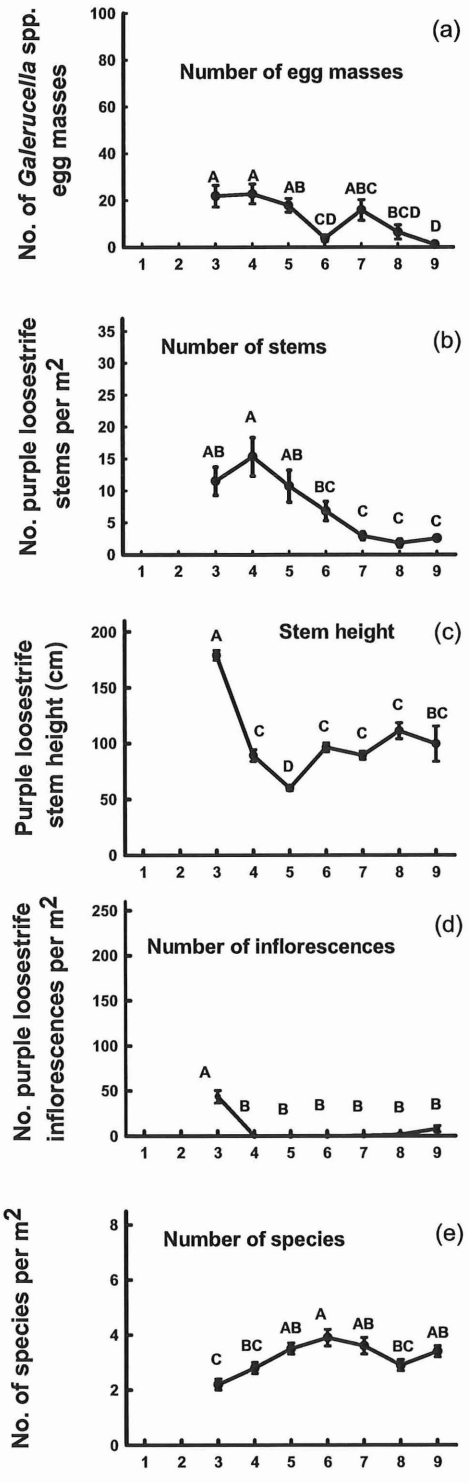

Fig. 1. Density of *Galerucella* spp. egg masses (a) and effect on purple loosestrife stem density m^2 (b), purple loosestrife stem height (c), purple loosestrife flowering (d), and number of plant species other than purple loosestrife (e), by year after release (Circle Lake, Minnesota). Within each figure, means with the same letter are not significantly different as determined by Ryan-Einot-Gabriel-Welsch Multiple Range Test ($P<0.05$). Error bars are ± standard error about the mean.

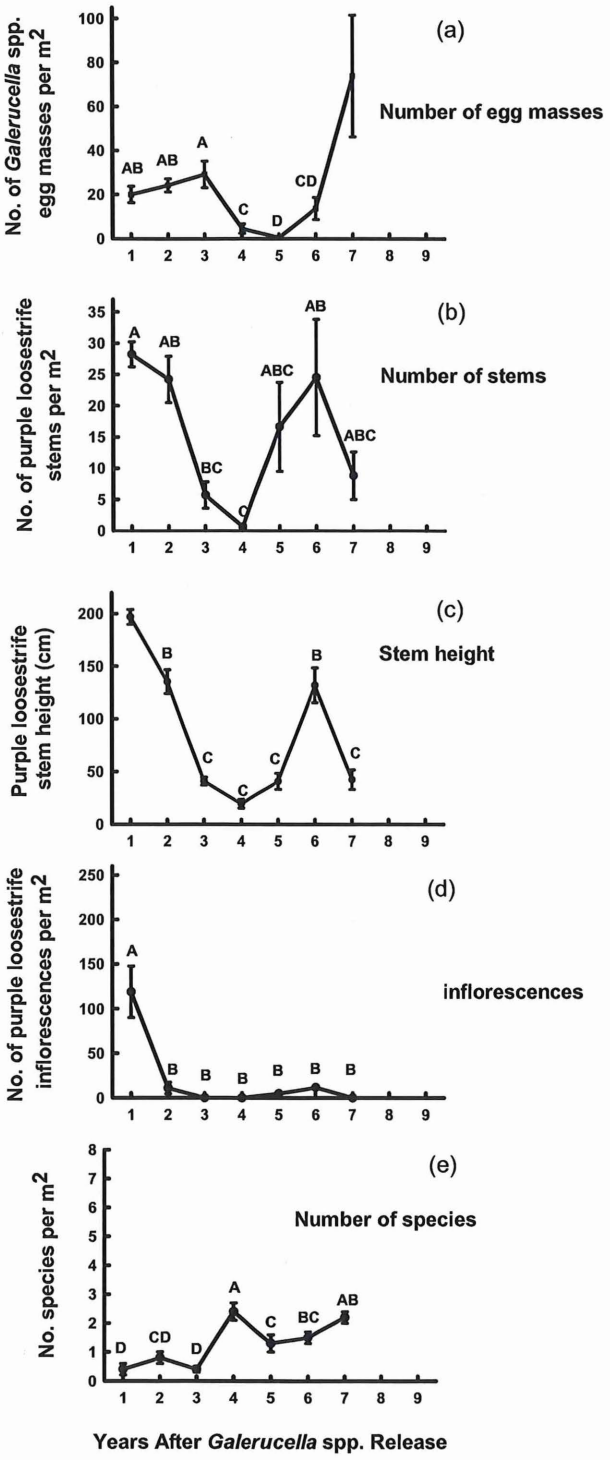

Fig. 2. Density of *Galerucella* spp. egg masses (a) and effect on purple loosestrife stem density m^2 (b), purple loosestrife stem height (c), purple loosestrife flowering (d), and number of plant species other than purple loosestrife (e), by year after release (Winona, Minnesota). Within each figure, means with the same letter are not significantly different as determined by Ryan-Einot-Gabriel-Welsch Multiple Range Test ($P<0.05$). Error bars are ± standard error about the mean.

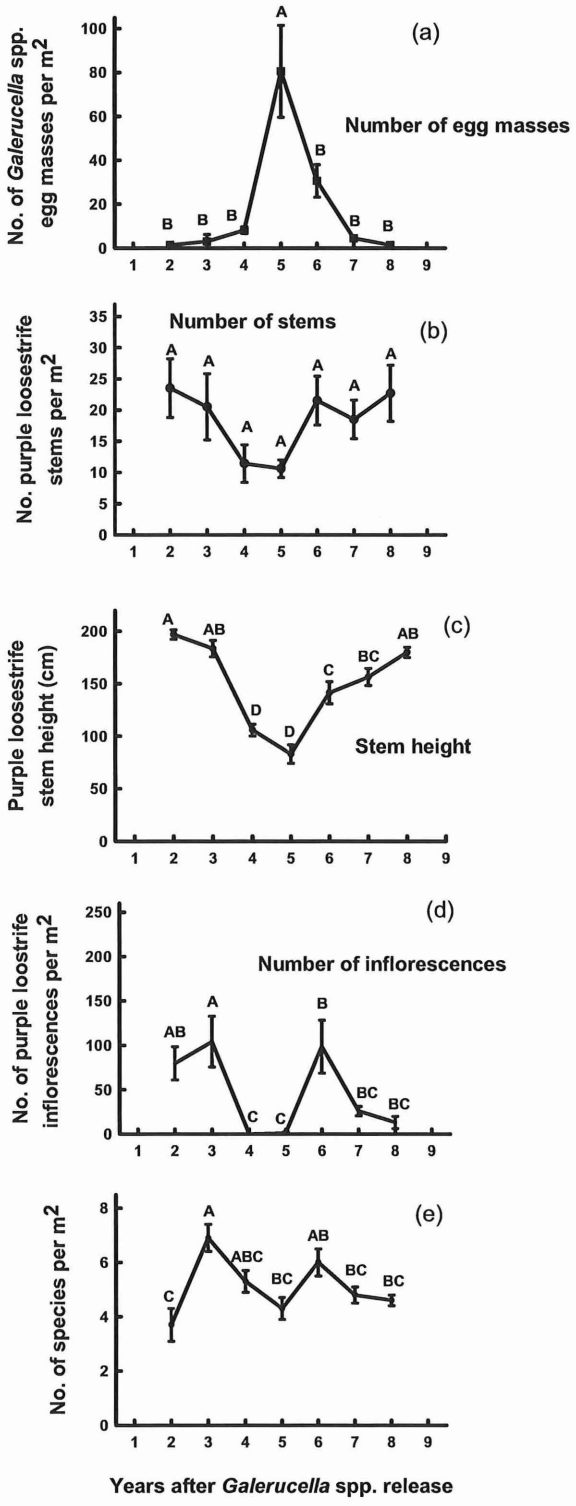

Fig. 3. Density of *Galerucella* spp. egg masses (a) and effect on purple loosestrife stem density m^2 (b), purple loosestrife stem height (c), purple loosestrife flowering (d), and number of plant species other than purple loosestrife (e), by year after release (White Bear Lake, Minnesota). Within each figure, means with the same letter are not significantly different as determined by Ryan-Einot-Gabriel-Welsch Multiple Range Test ($P<0.05$). Error bars are ± standard error about the mean.

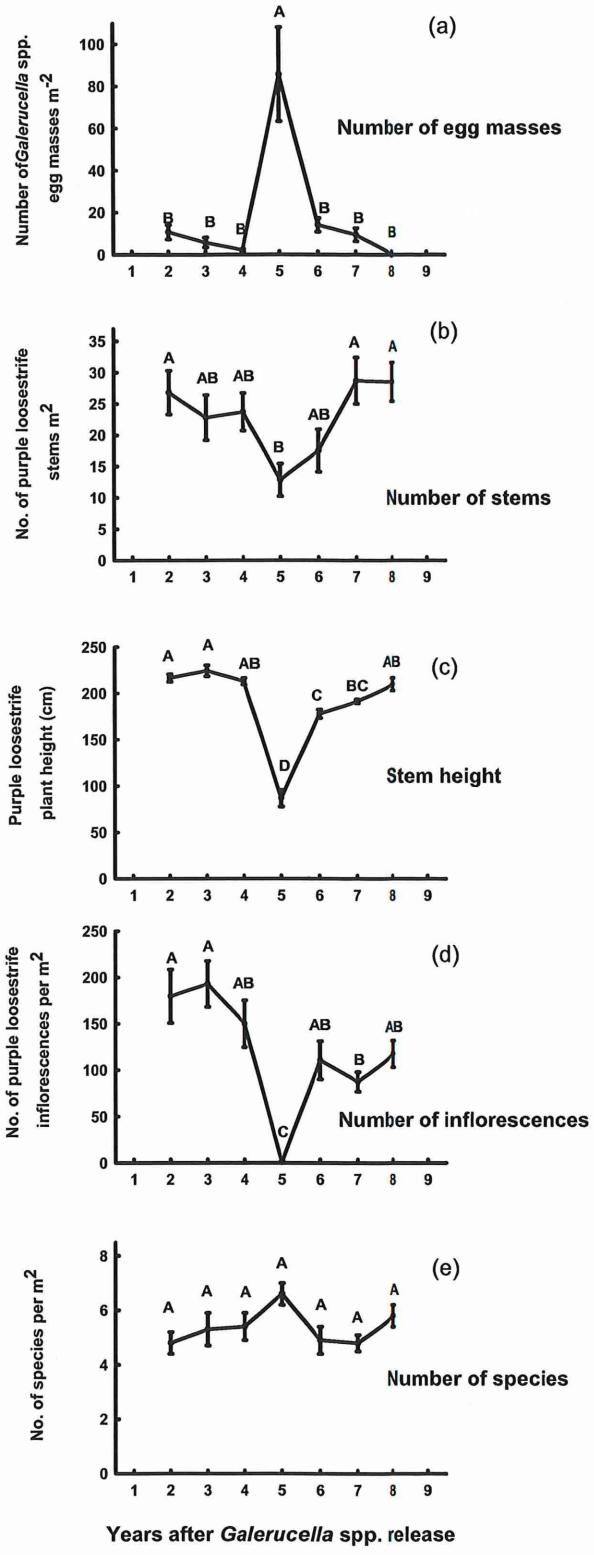

Fig. 4. Density of *Galerucella* spp. egg masses (a) and effect on purple loosestrife stem density m^2 (b), purple loosestrife stem height (c), purple loosestrife flowering (d), and number of plant species other than purple loosestrife (e), by year after release (Reno, Minnesota). Within each figure, means with the same letter are not significantly different as determined by Ryan-Einot-Gabriel-Welsch Multiple Range Test ($P<0.05$). Error bars are ± standard error about the mean.

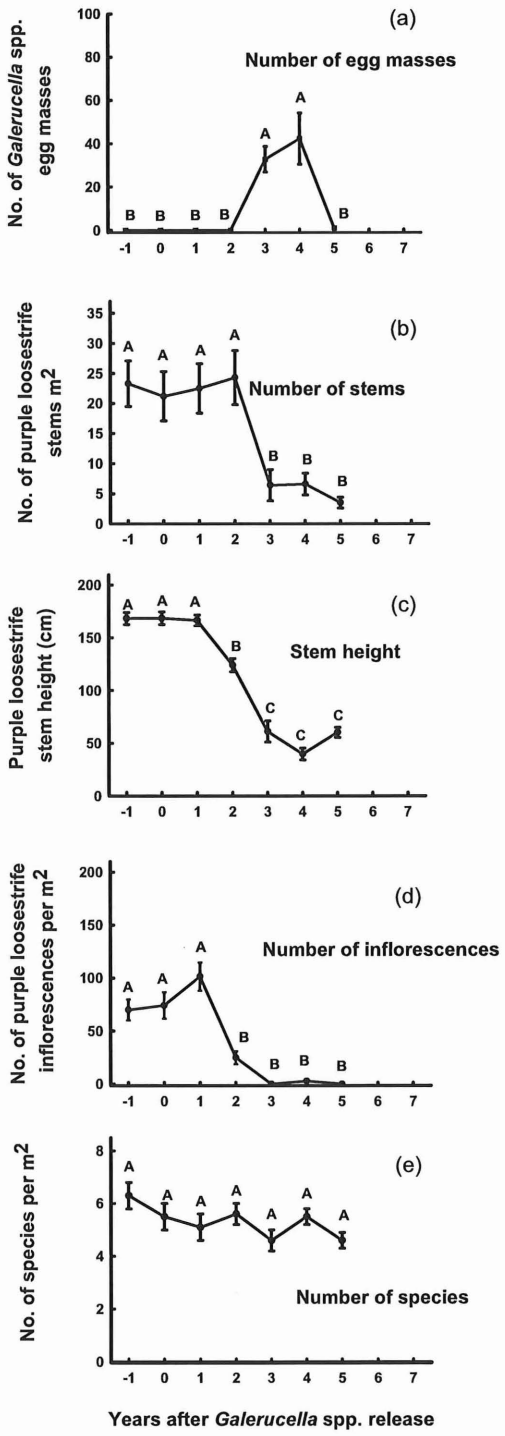

Fig. 5. Density of *Galerucella* spp. egg masses (a) and effect on purple loosestrife stem density m^2 (b), purple loosestrife stem height (c), purple loosestrife flowering (d), and number of plant species other than purple loosestrife (e), by year after release (Big Marine Lake, Minnesota). Within each figure, means with the same letter are not significantly different as determined by Ryan-Einot-Gabriel-Welsch Multiple Range Test ($P<0.05$). Error bars are ± standard error about the mean.

Final Report for Result 2, Activity 2
(2001 LCMR- Biological Control of Eurasian Watermilfoil and Purple Loosestrife-Continuation)

Growth and phenology of three Lythraceae species in relation to *Galerucella* spp.

BY

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Abstract

Previous studies have characterized the feeding, oviposition and larval development of the biological control insects, *Galerucella* spp., on non-target Lythraceae species, including two species native to Minnesota, winged loosestrife (*Lythrum alatum*) and swamp loosestrife (*Decodon verticillatus*). However, the impact of *Galerucella* spp. feeding on growth and seed production of the non-targets, winged loosestrife and swamp loosestrife, has not been reported. The objective of this study was to compare the phenology, growth and seed capsule production of winged loosestrife and swamp loosestrife, in relation to purple loosestrife (*Lythrum salicaria*), with and without the impact of *Galerucella* spp. Our study has documented minimal larval feeding on winged loosestrife and swamp loosestrife from the first generation of beetles in mid-June. Although *Galerucella* larvae were present on swamp and winged loosestrife, with one exception, none of the measured plant growth or reproductive parameters were reduced as a result of larval or adult *Galerucella* feeding. In the first year of the study, number of winged loosestrife seed capsules were reduced with *Galerucella* feeding compared to control plants. However, there were no *Galerucella* spp. present on winged loosestrife in the second year of the study. In Minnesota, flowering and seed development in swamp loosestrife occurs a month later than in purple loosestrife or winged loosestrife. Since *Galerucella* larval shoot tip feeding reduces the number of seed capsules formed on purple loosestrife, missing the main period of larval feeding in mid-June provides a degree of "phenological protection" for swamp loosestrife from *Galerucella* spp. feeding.

Introduction

Host specificity screening for potential weed biological control agents is designed to determine whether a potential biological control insect can complete its life cycle on a non-target plant in a no-choice testing system (McEvoy 1996). Prior to release of *Galerucella* spp. in North America for the biological control of purple loosestrife (*Lythrum salicaria*), host specificity tests were conducted in Europe and the United States (Kok et al. 1992, Blossey 1994). Results of the tests indicated that *Galerucella* spp. fed and oviposited on several species of *Lythrum*, including two species native to Minnesota, winged loosestrife (*Lythrum alatum*) (Blossey 1994, Kok et al. 1992) and swamp loosestrife (*Decodon verticillatus*) (Kok et al. 1992). However, the only non-target species that supported *Galerucella* larval development past the first instar was winged loosestrife (Blossey 1994, Kok et al. 1992).

Target and nontarget plants. Purple loosestrife is a perennial emergent wetland plant introduced to North America from Europe (Thompson et al. 1987). Purple loosestrife displaces valuable wetland plant species and is an extremely successful colonizer of disturbed wetland ecosystems (Thompson et al. 1987). This species is a herbaceous perennial and forms a woody crown from which new shoots emerge every year (Shamsi and Whitehead 1973). Seed dispersal, rather than vegetative reproduction is the major means of dissemination. It is estimated that each plant is capable of producing up to 2.7 million seeds per season (Thompson et al. 1987). The prolific seed production and subsequent seed rain leads to the creation of an extensive seed bank (Welling and Becker 1990). Once a seedbank is established, purple loosestrife more successfully colonizes disturbed and open sites than do native species (Welling and Becker 1993).

In North America, the most cosmopolitan native species of *Lythrum* is winged loosestrife, *Lythrum alatum*, which grows throughout the United States and Canada (Blackwell 1970; Cody 1978; Graham 1975). Winged loosestrife flowers are distylous (have two flower morphs) (Anderson et al. 1993b) and are also pollinated by large insects such as bees and butterflies (Levin 1970). Winged loosestrife grows to 1.0 m in height and may be distinguished from purple loosestrife by having one flower per leaf axil (Graham 1975). Winged loosestrife is often found growing in drier sites than purple loosestrife, although both species can inhabit the same wetland (Anderson and Ascher 1993a).

Swamp loosestrife or water willow (*Decodon verticillatus*) is also a North American native plant of the Lythraceae family and grows north to Canada and as far south as Louisiana Swamp loosestrife is a perennial species, tristylous and is self-compatable (Eckert and Barrett (1993). It is estimated that 30% of the progeny are the result of self-fertilization. Swamp loosestrife plants also reproduce vegetatively when stems contact moist soil and produce new shoots and adventitious roots (Eckert and Barrett 1993). This species grows in aquatic habitats similar to purple loosestrife. Shoots of swamp loosestrife exhibit an arching growth habit and flowers are arranged in dense clusters in leaf axils (Gleason 1952). Swamp loosestrife is classified as a species of special concern in Minnesota (Minnesota Dept. Nat. Resources 1996).

In addition to potential concerns of the effect of introduced biological control insects on related nontarget plants, concern exists regarding the possible deleterious effects of purple loosestrife itself on closely related native plants. Purple loosestrife flowers are tristylous (have three flower morphs) and are self incompatible (Anderson and Ascher 1994). Flowers are pollinated by large insects such as bees. In a wetland study, purple loosestrife pollen was preferred over pollen from winged loosestrife flowers by both bees and butterflies (Levin 1970) Pollen transfer from purple loosestrife to winged loosestrife reduced seed set in winged loosestrife and commonly occurred in the field (Brown and Mitchell 2001, Brown et al. 2002).

As a preferred pollen source, purple loosestrife may have a competitive advantage over winged loosestrife beyond the effect of vegetative competition (Brown and Mitchell 2001).
Biological control insects. *G. calmariensis* and *G. pusilla* are two leaf -defoliating beetles(Chrysomelidae) with similar life histories (Blossey et al. 1995a) and in 1992, were introduced into North America from Europe as biological control agents for purple loosestrife. The beetles cause severe leaf and shoot defoliation through larval and adult feeding (Hight and Drea 1991). In Minnesota, overwintered adult *Galerucella* spp. emerge in mid-May to early-June, depending upon spring temperatures and begin feeding on developing shoots of purple loosestrife plants. Adults oviposit on leaves and stems in egg masses of approximately 5 eggs. After hatching, the first larval instar moves to the shoot meristem where it feeds on developing leaves through the second larval instar (McAvoy et al. 1997). Third instar larvae move out of the meristem and feed freely on fully expanded leaves where the feeding damage is characterized as "window-pane" damage by feeding on the leaf mesophyll while leaving the waxy cuticular layer intact (Hight and Drea 1991). Larval development from egg hatch to pupation typically takes about 30 days to complete. In Minnesota, by mid- to late- June, third instar larvae will descend to the ground and pupate in leaf litter on the ground or in aerenchymous root tissue if plants are in standing water. Adult *Galerucella* spp. emerge in early- to mid- July. A portion of the adults will feed on remaining purple loosestrife plants or on seedlings and soon begin laying eggs that will produce a second generation. In Minnesota, there is generally one generation of beetles per year (Loos and Ragsdale 1998) although a partial second generation is common in the southern one-third of the state. Some F_1 adults will not reproduce but rather feed on available plants and then enter reproductive diapause by late- July (Loos and Ragsdale 1998).

In the continental United States, non-target feeding by biological control insects on native plants is almost exclusively restricted to closely related target plants within the same genus (Pemberton 2000). Even when biological control insects do not form self-sustaining populations on non-target plants, spill-over damage may occur when non-target plants are near high populations of biocontrol insects (Schooler et al. 2003). For example, slight feeding and ovipostion on winged and winged loosestrife was noted in a field study in Canada and represented a "short term spill-over effect" (Corrigan et al. 1998). *Galerucella* spp. also fed and oviposited on another species of Lythraceae, crepe myrtle (*Lagerstroemia indica*), in field studies but larvae were not able to complete development. From these results, it was concluded that the release of *Galerucella* spp. posed little risk to crepe myrtle in North America (Schooler et al. 2003).

After evaluation, the Technical Advisory Group (TAG) determined that the benefit of introducing *Galerucella* spp. for the control of purple loosestrife outweighed the risk of potential feeding on populations of winged or swamp loosestrife (Blossey 1994). In the United States, *Galerucella* spp. were first approved for release for the biological control of purple loosestrife in 1992.

In screening potential biological control agents, examining the "physiological host range" of non-target hosts may not be sufficient. An examination of the "ecological host range", which includes non-target plant phenology and life cycle is also critical (McEvoy 1996, Louda et al. 2003). Previous studies have characterized the feeding, oviposition and larval development of *Galerucella* spp. on non-target plants (Blossey 1994, Kok 1992, Corrigan et al. 1998, Schooler et al. 2003). However, the impact of *Galerucella* spp. feeding on growth and seed production of the non-targets, winged loosestrife and swamp loosestrife, has not been reported. *Galerucella* spp. larvae feed on developing meristems of purple loosestrife. This results in production of

fewer seed capsules per inflorescence and fewer seeds per plant (Katovich et al. 2001) Phenological events, such as time of flowering and seed production, may provide an additional level of protection from non-target feeding. The objective of this study was to compare the phenology, growth and seed capsule production of two native species of Lythraceae, winged loosestrife and swamp loosestrife, in relation to purple loosestrife, with and without the impact of *Galerucella* spp.

Materials and Methods

Two studies were established in 2001, repeated in 2002 and were conducted at the St. Paul campus of the University of Minnesota. The first study was designed to determine the effect of *Galerucella* spp. on the growth and seed capsule production of purple loosestrife, winged loosestrife and swamp loosestrife. The second study was established to examine the phenology of *Galerucella* spp. in relation to the three Lythraceae species as well as phenological differences among the three plant species.

Effect of *Galerucella* spp. on growth and seed capsule production of winged, swamp and purple loosestrife. Winged, swamp and purple loosestrife seeds were planted in the greenhouse in a standard greenhouse mix (silt loam: sand: manure: peat, 1:1:1:1, v/v/v/v) in early spring of 2000 and 2001. In July 2000, plants of all three species were transplanted outside into individual mesocosms (plastic wading pools, 0.9m diameter and 0.2 m depth) and filled with a peat based potting mix. Eight plants of a single species were transplanted into individual mesocosms for a total of 2 mesocosms of each species and were placed in a random arrangement. The plants grew through the season and were overwintered to establish plants for treatment the following year. For overwintering, all plants were mulched with straw and wood chips to simulate the natural insulative cover in wetlands. In the spring of 2001, the following treatments were applied for each species; 1) a control where all plants in one mesocom were treated with the systemic insecticide, imidacloprid, to prevent *Galerucella* spp. feeding and 2) allowing feral *Galerucella* spp. to feed and oviposit on all plants in a free choice fashion. The experiment was repeated with a new set of plants that were planted outside in pools in July 2001. The second experiment was initiated in the spring of 2002. In May 2002, few feral *Galerucella* adults were present on *Lythrum* plants in the experiment. This may have been due to the removal of a reservoir of beetles from established purple loosestrife plants growing in an adjacent area that were removed for building construction. For this reason, approximately 700 *Galerucella* spp. adults were collected from a wetland and released on the periphery of the study area. The beetles were able to freely locate potential host plants and lay eggs. The amount of adult and larval feeding and number of egg masses were recorded for each plant species. At the end of the growing season, shoot dry weights were obtained and number of seed capsules were counted on one randomly selected inflorescence from each plant. In 2001, the number of seed capsules were counted on an inflorescence from four plants in each mesocosm. In 2002, an inflorescence from all eight plants in each mesocom were counted. The experiment was a randomized complete block design in a split plot arrangement with insecticide or no insecticide treatment as the main plot and plant species as subplot. Each treatment was replicated eight times with each replication being a single plant. Analysis of variance was performed on data and means were separated with a protected Least Significant Difference test. Data was tested, found to be homogenous and was not transformed.

Phenology of *Galerucella* spp., purple loosestrife, winged loosestrife and swamp loosestrife study. Wetland mesocosms were created so that all three species were grown under the same environmental conditions. Winged, swamp and purple loosestrife seeds were planted in the greenhouse and plants were transplanted into outdoor mesocosms the year preceding treatment as described in the previous experiment. Mesocosms were dug into the ground so that soil temperature was not altered by aboveground placement. Plants were placed in a random arrangement with all three species present in a single mesocosm, for a total of nine plants per pool, three of each species. In the fall, plants were mulched lightly and overwintered. Each mesocosm was replicated four times.

Beginning in April, 2001, date of shoot emergence was noted and number of crown buds were recorded for each plant on a weekly basis. Date of flower bud formation and flowering was also recorded for each plant. Date of adult *Galerucella* emergence was noted. In early June, the number of *Galerucella* egg masses was recorded for each plant as well as date of first larval feeding. Air temperatures were obtained from the University of Minnesota Climate Center. Growing degree days (GDD_{b10}) were estimated using a base air temperature of 10 C with no maximum temperature (Climatologic Working Group 2001). Although a base temperature for purple loosestrife is not described in the literature, base temperatures from other perennial species such as alfalfa and hemp dogbane were used as a point of reference (Sharratt et al. 1989; Ransom et al. 1998). The experiment was analyzed as a randomized complete block design. Data was subjected to Analysis of Variance and means separated with a Least Significant Difference test. Data was tested and found to be homogeneous and was not transformed. The experiment was repeated in 2002 with a new set of plants, which were planted outside in mesocosms in the July, 2001.

Results and Discussion

Effect of *Galerucella* spp. on growth and seed capsule production of winged, swamp and purple loosestrife study. Number of *Galerucella* spp. egg masses in early June of 2001 and 2002 was highest on purple loosestrife plants with an average egg mass counts of 120.4 and 123.0 per plant for 2001 and 2002 respectively (Table 1). Both winged and swamp loosestrife had significantly fewer egg mass counts. There were an average of 17.5 and 0 egg masses per plant in 2001 and 2002 respectively on winged loosestrife plants. Swamp loosestrife plants had an average of fewer than one egg mass present for both years.

All but one of the end of season parameters measured for purple loosestrife were reduced as a result of *Galerucella* spp. feeding compared with the insecticide treated control (Table 2). Aboveground shoot biomass, plant height and number of seed capsules were reduced as a consequence of *Galerucella* feeding. The number of shoots at the end of the season was higher in plants with *Galerucella* feeding. This was most likely due to the release of crown buds as a result of diminished main shoot apical dominance caused by shoot defoliation.

There were no differences in dry weights of winged loosestrife shoots, plant height or number of shoots at the end of the season with or without *Galeurcella* feeding. In 2001, the number of seed capsules per inflorescence were reduced on plants with *Galerucella* feeding as compared to the insecticide control. However, in 2002, there were no differences in the number of seed capsules between treatments. In 2001, egg masses were present on 88% of winged loosestrife plants. In 2002, egg masses were not present on any winged loosestrife plants. The reason why egg masses were present on winged loosestrife plants in 2001 and not in 2002 is not known. However, in 2002, a different source of beetles were used in the study.

In both years of the experiment, swamp loosestrife plants had an average of fewer than one *Galerucella* egg mass per plant and little, if any, larval feeding damage. End of season shoot dry weight, number of shoots and seed capsules did not differ between the two treatments. Plant height was the only parameter which differed between treatments. Plants exposed to *Galerucella* spp. were shorter than the insecticide control plants. However, since there was little, if any non-target feeding visible on these plants, feeding by *Galerucella* does not appear to be the cause of the shorter plants.

Phenology of *Galerucella* spp. in relation to that of purple loosestrife, winged loosestrife and swamp loosestrife. In the spring of 2001 and 2002, the average date of purple loosestrife shoot emergence from crown buds occurred on April 17 when the average number of accumulated GDD_{b10} was 37 (Table 3). Shoots of winged loosestrife and swamp loosestrife emerged later than purple loosestrife shoots and were first observed on May 10 and May 16 respectively. At this time, accumulated GDD_{b10} were 178 and 211 for winged loosestrife and swamp loosestrife respectively. It is not known whether the spring emergence of shoots from crown buds of winged or swamp loosestrife is influenced by temperature or photoperiod as in other perennial species (Becker and Fawcett 1998). Number of shoots emerging from crown buds was notable higher for purple loosestrife plants than the other species (Figure 1). Additionally, the rate of shoot emergence from crown buds was notably faster for purple loosestrife, indicating the early resource capture of light and the potential for site domination of purple loosestrife.

The initial date of purple loosestrife flowering varies among regions and among populations within regions (Olsson and Agren 2002). In our study, flower buds were first observed on purple loosestrife on June 6 in 2001 (Table 3). In 2002, all purple loosestrife shoots were defoliated by *Galerucella* spp. so flowering was delayed. However, past studies show similar purple loosestrife flowering dates in Minnesota (Katovich et al. 1998). Purple loosestrife plants requires a critical day-length of 13 h for flower initiation and stem elongation to occur (Shamsi and Whitehead 1973). In St. Paul, MN (latitude 44° 99' N, longitude 93° 21' W, 280 m above sea level) a 13 h daylength was reached on April 5, 2001 and April 6, 2002. This means that a critical daylength of 13 h was reached prior to emergence of crown buds from the soil in the spring. Flowering at the latitude of St. Paul, MN is probably not triggered by a change in daylength as the critical daylength was achieved approximately two months prior to crown bud emergence in the spring.

As seen for shoot emergence from crown buds, initiation of flower buds occurred later in winged loosestrife and swamp loosestrife compared with purple loosestrife. (Figure 2). Flower buds were first observed in winged loosestrife on June 23 (813 GDD_{b10}) and on July 18 for swamp loosestrife (1481 GDD_{b10}) compared to 510 GDD_{b10} for purple loosestrife. Date of flowering of the three species was defined as the time when the first flowers had opened. Purple loosestrife has an indeterminate inflorescence and flowering occurs until the end of the growing season. The first completely opened flowers were first noted for purple loosestrife and winged on June 28 (995 GDD_{b10}) and June 30 (1035 GDD_{b10}) respectively. However, flowers did not open in swamp loosestrife until August 2 (1876 GDD_{b10}). The date of the first fully opened flowers in swamp loosestrife occurred a full month later than the other two species.

Galerucella spp. adult feeding was first observed in all three species in late May (May 23 for purple loosestrife and winged loosestrife and May 27 for swamp loosestrife). Egg masses were present about a week later (Figure 2). First instar larval feeding was first observed in the middle of June on all three plant species and F_1 adults had emerged by July 11. Similar

phenologies of *Galerucella* spp. life stages were recorded by Lindgren in Manitoba (2003).

As expected, larval feeding by *Galerucella* spp. resulted in fewer seed capsules on purple loosestrife inflorescences because of shoot tip and flower bud damage (Katovich et al. 2001). Under Minnesota climatic conditions, there is usually one generation of beetles per year (Loos and Ragsdale 1998). Swamp loosestrife plants flowered and set seed in August (Figure 2). The delayed phenological development of swamp loosestrife plants, compared with the other two species, resulted in avoidance of the first and second larval instar shoot tip feeding damage caused by the first generation of *Galerucella* spp. beetles (Figure 2). As a result, the shoot meristems and developing flower buds of swamp loosestrife were not damaged as they missed the larval damage that could have resulted in a reduction in number of seed capsules. In regions south of St. Paul, MN, *Galerucella* may produce more than one generation of beetles per year. Flowering in swamp loosestrife may coincide with later generations of *Galerucella* spp. larval feeding.

Differences in growth among Lythraceae species. Absent *Galerucella* spp., plant height in mid- July was greatest for purple loosestrife (Table 4). Total plant dry weight at the end of the season was approximately four times greater for purple loosestrife than the other species. Also, number of seed capsules averaged 239 capsules per inflorescence for purple loosestrife verses 75 and 23 capsules per inflorescence for winged loosestrife and swamp loosestrife, respectively. Estimates of purple loosestrife seed production per plant range from 600,000 (Cutright 1986) to 2,000,000 (Thompson et al 1987). Brown et al. (2002) determined that each winged loosestrife seed capsule produced 63 seed. From this, we estimate that each winged loosestrife plant produced approximately 147,000 seeds per plant in our study. Dorken and Eckert (2001) found an average of 1139 seeds per plant in swamp loosestrife. Our results show that if winged loosestrife or swamp loosestrife were growing the same wetland with purple loosestrife, purple loosestrife would produce considerably more seed per plant per year than the native species. This would eventually overwhelm all other plant competitors through seedling recruitment, as shown by Welling and Becker (1990) who estimated that 400,000 purple loosestrife seed m^{-2} were present in the upper 5 cm of a Minnesota wetland.

Initial host specificity studies with the native non-target species, winged loosestrife and swamp loosestrife demonstrated that *Galerucella* spp. would feed, oviposit, and in the case of winged loosestrife, larvae would develop to the first instar stage (Blossey 1994, Kok et al. 1992). Our study has documented minimal larval feeding on winged loosestrife and swamp loosestrife from the first generation of beetles in mid-June. Although *Galerucella* larvae were present on swamp loosestrife, none of the measured plant growth or reproductive parameters were reduced as a result of larval or adult *Galerucella* feeding. In addition, in Minnesota, flowering and seed development in swamp loosestrife occurs a month later than in purple loosestrife or winged loosestrife. Since *Galerucella* larval shoot tip feeding reduces the number of seed capsules formed on purple loosestrife (Katovich et al. 2001), missing the main period of larval feeding in mid-June provides a degree of "phenological protection" for swamp loosestrife from *Galerucella* spp. feeding.

In the first year of our study, the number of seed capsules were reduced by 31% on winged loosestrife plants compared with an insecticide treated control. No other plant growth parameters were reduced. However, in the second year of our study, no *Galeucella* beetles or egg masses were present on winged loosestrife plants the entire season and number of seed capsules were not reduced. By contrast, with *Galerucella* spp. feeding, there was a 64% reduction of the number of seed capsules produced by purple loosestrife. In a wetland where

70% of purple loosestrife leaves where defoliated by *Galerucella*, few to no purple loosestrife flower buds and seeds were produced (Katovich et al. 2001). Thus, the potential exists for a great reduction of purple loosestrife seeds by *Galerucella* spp. feeding with little or no reduction in the native, nontarget Lythraceae.

Purple loosestrife is a highly competitive plant compared with winged and swamp loosestrife. This is evident from phenological characteristics, such as earlier spring emergence, and greater number of shoots emerging from crown buds in the spring. Other growth traits, such as greater plant height and above ground biomass were higher in purple loosestrife, compared with swamp or winged loosestrife, and have been correlated with a greater competitive ability in purple loosestrife (Gaudet and Keddy, 1988). Purple loosestrife also has a greater potential for seed production compared with the other two plant species and Weihe and Neely (1977) found that the number of reproductive structures was an indicator of competitive success in purple loosestrife. Due to the highly competitive growth characteristics of purple loosestrife, it may be argued that there is a greater benefit from release of *Galerucella* in wetlands compared with the minimal non-target feeding and ovipostion effects on winged loosestrife and swamp loosestrife.

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Table 1. Number of *Galerucella* spp. egg masses present on three species of Lythraceae, June 2001 and 2002. St. Paul, MN

Species	Number of *Galerucella* spp. egg mass	
	2001	**2002**
Purple loosestrife	120.4	123.0
Winged loosestrife	17.5	0
Swamp loosestrife	0.8	0.3
(LSD 0.05)	26.7	42.2

Table 2. Shoot dry weights, plant heights, number of shoots and seed capsules of three species of Lythraceae with and without *Galerucella* spp. feeding, St. Paul, MN, 2001 and 2002.

Feeding status	End of season shoot dry weight	End of season plant height	Seed capsules		Shoots
Purple loosestrife	(g)	(cm)	(no.)		(no.)
no *Galerucella* feeding	86.9	150.3	258.8		7
with *Galerucella* feeding	56.3	104.6	92.9		11.1
LSD (0.05)	28	12.8	56.3		3.7
Swamp loosestrife					
no *Galerucella* feeding	21.8	114.6	21.1		5.5
with *Galerucella* feeding	30.6	84.9	28.6		5.5
LSD (0.05)	NS	28	NS		NS
Winged loosestrife			2001	2002	
no *Galerucella* feeding	12.2	62.8	105	48.5	31.1
with *Galerucella* feeding	10.2	54.9	72.3	55	28.3
LSD (0.05)	NS	NS	24.2	NS	NS

Table 3. Crown bud emergence time, initiation of flower buds and date of first flowering, 2001 and 2002.

	Purple loosestrife			Winged loosestrife			Swamp loosestrife		
	Date	Julian Date	GDD_{b10}	Date	Julian Date	GDD_{b10}	Date	Julian Date	GDD_{b10}
emergence	4-17	107	37	5-10	130	178	5-16	136	211
flower bud	6-6[1]	157	510	6-23	174	813	7-18	199	1481
flowering	6-28[1]	179	995	6-30	182	1035	8-2	214	1876

[1]Based on 2001 results only

Table 4. Growth differences among species of Lythraceae

Species	Plant Height	Numbers of stems	Number of seed capsules per inflorescence	End of season dry weight
	(cm)			(g)
Purple loosestrife	105	7	239	80
Winged loosestrife	70	31	75	15
Swamp loosestrife	61	6	23	21
LSD (0.05)	14	9	26	8

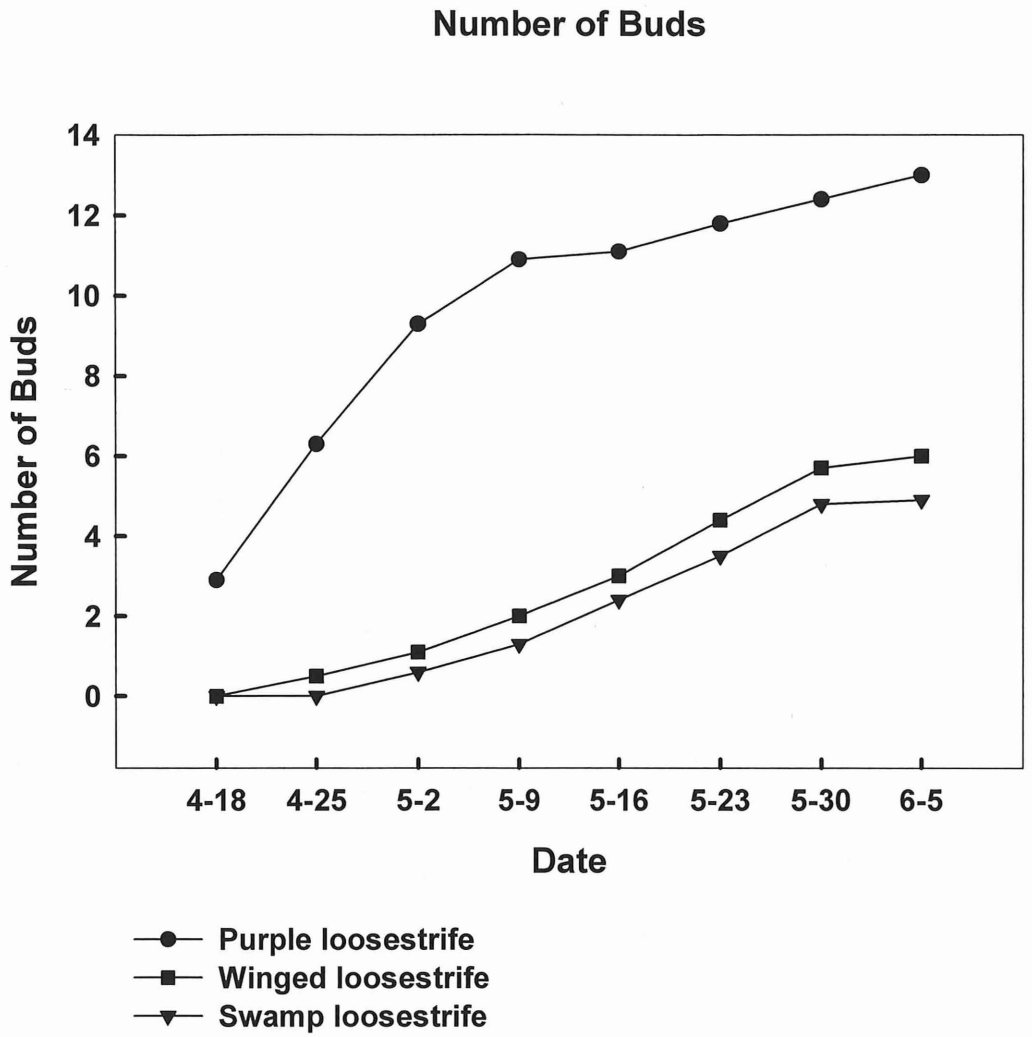

Figure1. Number of shoots emerging from crown buds for purple, winged and swamp loosestrife.

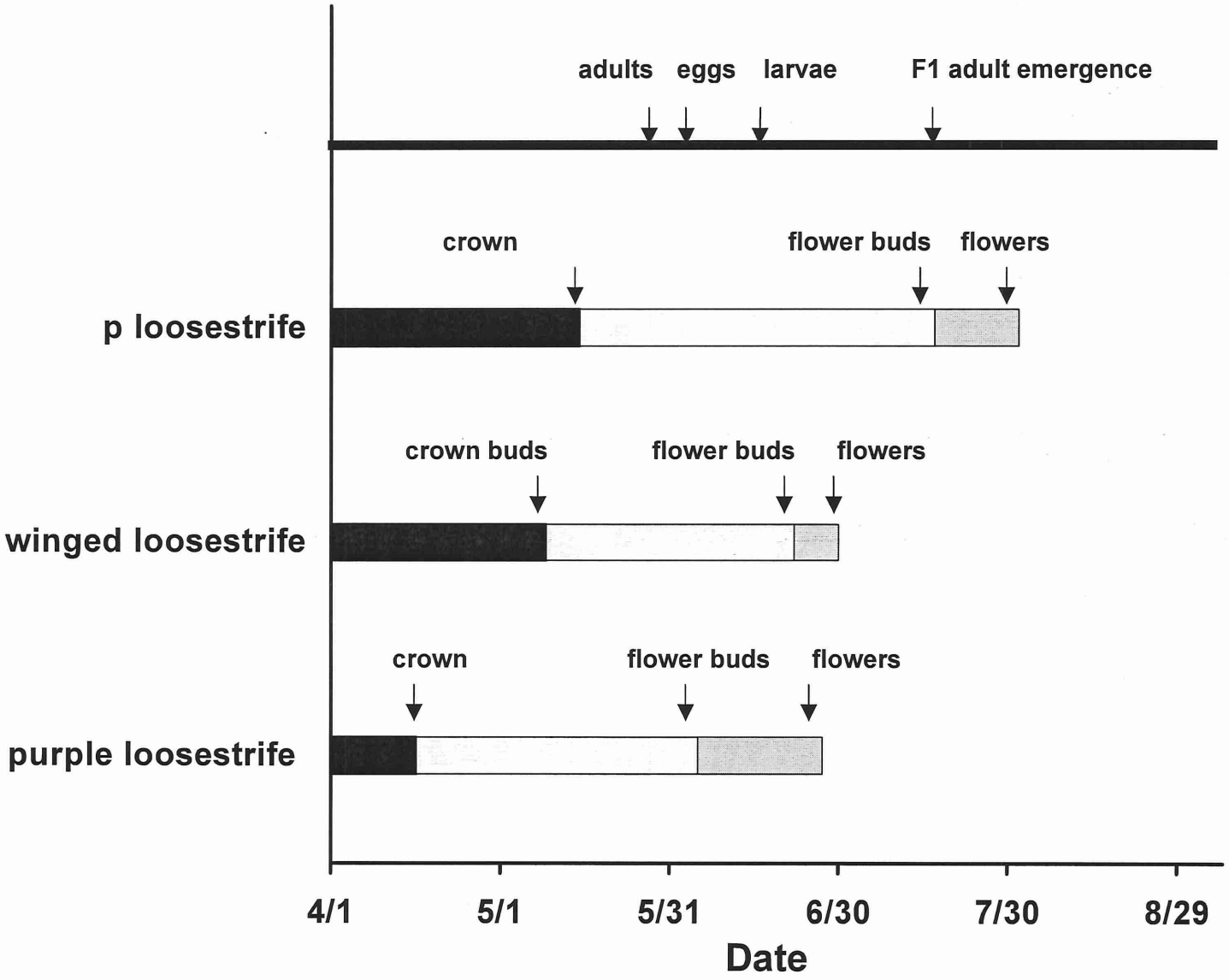

Figure 2. Phenology of purple, winged and swamp loosestrife, and the leaf-feeding beetle, Galerucella spp.