INTERACTIVE EFFECTS OF PREDATION, PLANT QUALITY AND HERBIVORE MOVEMENT ON THE SUCCESS OF THE PURPLE LOOSESTRIFE BIOCONTROL PROGRAM

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INTERACTIVE EFFECTS OF PREDATION, PLANT QUALITY AND HERBIVORE MOVEMENT ON THE SUCCESS OF THE PURPLE LOOSESTRIFE BIOCONTROL PROGRAM

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The leaf-feeding beetles *Galerucella calmariensis* and *G. pusilla*, introduced as biocontrol agents of the invasive purple loosestrife, are usually highly effective in controlling purple loosestrife. However, at certain sites the insects fail to attain outbreak populations, possibly due to the interaction of predation and flooding regime. Predator exclusion experiments indicated a positive effect of flooding on leaf-beetle survival for all developmental stages, while predator exposure had little effect. The effect of predation did not vary between regions with successful and lack of purple loosestrife control. Purple loosestrife plants grown in flooded soils had higher water content and lower tannin concentration than plants grown in well-drained soil, likely indicating that flooding improves purple loosestrife nutritional quality for leaf-beetles.

Dispersal behavior may also affect leaf-beetle dynamics and hence purple loosestrife control. The effect of matrix habitat, isolation and plant damage on leaf-beetle movement was evaluated through a mark-recapture/resight experiment. Leaf-beetles were more likely to colonize purple loosestrife patches embedded in meadow than in forest habitat and they preferred previously attacked purple loosestrife plants over un-attacked plants, especially in the forest habitat. Movement of both species was similarly

affected by matrix type, but *G. pusilla* recaptures were always higher than *G. calmariensis*. Additionally, I used a mark-recapture study and a spatially-explicit simulation model to evaluate the effect of host-plant damage and distribution, and presence of conspecifics on the dispersal behavior of *Galerucella* spp. In the field, leaf-beetles preferred purple loosestrife plants that had been previously attacked by conspecifics. A factorial simulation experiment with degree of host-plant aggregation and insect density as factors revealed that mean dispersal distances were lower in the least aggregated landscape, but were not affected by insect density. Dispersal kernels for all cases were fat tailed but kurtosis was higher for the least aggregated landscape.

Overall, our results indicate that environmental disturbances such as flooding and landscape features such as matrix habitat mediate *Galerucella*'s interactions and behavior, leading to variations in their survival and distribution. Biotic and abiotic interactions of biocontrol agents in the introduced range may influence the success of a biocontrol program and should be considered in management.

BIOGRAPHICAL SKETCH

Andrea Dávalos was born and raised in Quito, Ecuador. She graduated in 1997 with a Bachelor of Science degree in Biology from the Pontificia Universidad Católica del Ecuador. After graduation, she worked for EcoCiencia, an environmental Ecuadorian NGO monitoring the ecological impacts of timber harvesting in the Chocó Region of Northwestern Ecuador. Her interests in ecological research and resources management prompted her to pursue Cornell's Natural Resources Master's Program. After completing her Master's Andrea decided that she was not quite ready to leave Cornell and started a PhD program in the Ecology and Management of Invasive Plants Program in the Department of Natural Resources at Cornell. Andrea is now residing in Brazil.

A Nicolas, Felipe y Thomas

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CHAPTER 1

FLOODING AFFECTS HOST PLANT QUALITY AND PREDATION: CONSEQUENCES FOR PURPLE LOOSESTRIFE LEAF-BEETLES

Abstract

Environmental stress, such as flooding, may affect the relative strength of top-down and bottom-up forces influencing herbivore dynamics. We investigated the effect of flooding on both top-down (predator) and bottom-up (plant quality) forces operating on two leaf-beetles (*Galerucella calmariensis* and *G. pusilla*) released in North America as biological control agents of purple loosestrife (*Lythrum salicaria*). Predation and flooding regime have been linked to low *Galerucella* recruitment at sites where the insects failed to attain outbreak populations. Predator exclusion experiments at adjacent flooded and non-flooded sites indicated a positive effect of flooding on leaf-beetle survival for all developmental stages, while predator exposure had little effect. The effect of predation was similar in sites with successful and failed purple loosestrife control. The effect of flooding on purple loosestrife plant quality was evaluated in a common garden study where plants were grown under different flooding treatments. Plants grown in flooded treatments had higher water content and lower tannic acid concentration than plants grown in well-drained soil. Consistent with field observations, leaf-beetle oviposition rate and survival were higher on flooded plants. Results indicate that both bottom-up and top-down forces operate on *Galerucella* populations, yet their relative strength is mediated by flooding regime.

Introduction

Herbivore abundance show spatial variation, even when their host plant is widely distributed and available (Hanski 1999). Multiple factors may cause this variation, but special attention has been placed on bottom-up (host plant quality and quantity) and top-down (effects of natural enemies) forces on herbivore populations (Hairston et al. 1960, Hunter and Price 1992, Forkner and Hunter 2000, Schmitz et al. 2000). Although both mechanisms were traditionally viewed as independent forces, it is now recognized that they can act simultaneously (Hunter and Price 1992, Hawkins 2001). Recent emphasis has been on identifying the factors that mediate relative contributions of host plant quality and natural enemies on herbivore dynamics, and how they vary in space and time (Denno et al. 2005, Gripenberg and Roslin 2007).

It has been proposed that the strength of top-down and bottom-up forces varies along environmental stress gradients: in stressful environments, biotic forces such as predators are less abundant and thus abiotic factors are more important, while in benign environments where predators are more common top-down forces become increasingly relevant (Menge and Sutherland 1976, Hunter and Price 1992, Preszler and Boecklen 1996). Flooding may reduce top-down effects by limiting predator dispersal and access of ground-dwelling arthropods to permanently flooded sites, thereby providing enemy free-space for herbivores. Additionally, plant adaptations to flooding may change host plant quality and influence herbivore and natural enemy dynamics.

Wetland plants develop several physiological and morphological adaptations to cope with the anoxic environment produced by flooding, such as the development of aerenchyma, adventitious roots and elongated shoot

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internodes (Blom and Voesenek 1996, Voesenek et al. 2006). Flooding may also change plant biomass allocation and architecture (Miller and Zedler 2003, Fraser and Karnezis 2005), decrease photosynthetic rates (Voesenek et al. 2006), and may disrupt nutrient uptake and thereby decrease leaf nitrogen and protein concentrations (Kozlowski 2000, Güsewell et al. 2003). Moderate stress produced by flooding or minor drought may impose constraints on plant growth favoring production of plant defenses (Herms and Mattson 1992). Although increased production of plant defenses has been observed under minor drought (Herms and Mattson 1992, English-Loeb et al. 1997), the effects of flooding on secondary metabolites production is still poorly understood (Lower et al. 2003).

Changes in abiotic conditions and variations in plant morphology, performance and chemistry produced by flooding may alter the arthropod community structure, creating a spatial mosaic along which herbivore pressure and top-down control vary (Nakamura et al. 2005, Elderd 2006). For example, flooding not only increases performance of the monkey flower, *Mimulus gutattus*, but herbivore effects are stronger in non-flooded areas where grasshoppers (Orthoptera) are abundant compared to flooded areas where leafhoppers (Homoptera: Cicadellidae) are more common (Elderd 2006). Similarly, *Galerucella lineola* F. adults show strong affinity to moist habitats where their larvae perform best, leading to higher leaf-beetle abundance on *Salix phylicifolia* L. growing in flooded areas compared to plants growing in well-drained soils (Sipura et al. 2002).

The objective of this study was to evaluate the role of flooding on the relative importance of top-down and bottom-up forces operating on two specialist leaf-beetles *Galerucella calmariensis* L. and *G. pusilla* Duftschmid

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(Coleoptera:Chrysomelidae, hereafter referred to as *Galerucella*), introduced to North America to control the invasive purple loosestrife (*Lythrum salicaria* L.) (Malecki et al. 1993). Purple loosestrife is a wetland perennial able to thrive under a range of water availability (from permanently flooded to upland conditions). When plants are partially submerged aerenchymatous tissue and longer shoots develop, but overall plant biomass is not affected (Stevens et al. 1997, Lempe et al. 2001, Stevens et al. 2002). *Galerucella* have been widely released across North America attaining outbreak levels and significantly reducing *L. salicaria* abundance and performance at the majority of sites (Blossey and Skinner 2000, Blossey et al. 2001b). However, in some locations insects failed to establish or to increase population size to critical levels (Landis et al. 2003, Blossey and Nuzzo 2004, Denoth and Myers 2005, Grevstad 2006).

Predation has been proposed as the main reason for low *Galerucella* recruitment at drier sites (Landis et al. 2003, Blossey and Nuzzo 2004, Sebolt and Landis 2004), while field observations indicate that flooding may reduce biotic interference by natural enemies (Landis et al. 2003). The observation that *Galerucella* are more abundant at flooded sites compared with nearby drier sites led us to investigate factors that regulate *Galerucella* populations under different moisture regimes. Here we tested the following hypotheses: (1) Flooding decreases the effect of predation increasing *Galerucella* survival; (2) Predation is responsible for the inability of *Galerucella* to suppress purple loosestrife at certain sites; (3) Purple loosestrife plants grown in flooded soils exhibit traits associated with increased *Galerucella* performance; and (4) Flooding increases *Galerucella* performance and survival. We evaluated these hypotheses over three years using predator exclusion experiments in

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adjacent wetlands with different moisture regimes and in a common garden with plants grown under different water level treatments.

Methods

Study system

Purple loosestrife (*Lythrum salicaria*) is an invasive perennial wetland herb introduced to North America from Europe in the early 1800s (Thompson et al. 1987). Plants can grow across a wide moisture regime, but germination is limited to open moist soils where seedlings grow rapidly (> 1 cm/d) (Blossey 1995a). Mature plants can reach up to 2-3 m in height and develop 30-50 annual shoots that may produce 2.7 million seeds annually (Welling and Becker 1990). Plants regrow each year from the woody rootstock which serves as a storage organ (Thompson et al. 1987, Mal et al. 1992a, Malecki et al. 1993, Blossey 1995a, b). Purple loosestrife is rich in phenolic compounds (Rauha et al. 2001) but does not contain significant amounts of alkaloids (Steinfeld 1969, Fujita et al. 1972). Tannin concentration is particularly high in stems (6% dry weight) and leaves (24%) (Ma et al. 1996, Rauha et al. 2001). Purple loosestrife has aggressively invaded wetlands across North America, forming dense monospecific stands and causing negative ecological impacts on native plants, amphibians and bird communities (Whitt et al. 1999, Blossey et al. 2001b, Farnsworth and Ellis 2001, Brown et al. 2006). Conventional management methods failed to control purple loosestrife, leading to the development of a biological control program (Malecki et al. 1993, Hight et al. 1995, Blossey et al. 2001b).

Galerucella calmariensis and *G. pusilla* are two of the four host specific insects introduced from Europe to North America to control purple loosestrife.

Galerucella are sympatric specialist species that share similar life histories (Blossey 1995a). After adult emergence in early May, females mate and lay eggs on leaves and stems of purple loosestrife. Neonate larvae feed on young leaf tissue often concealed in apical buds, whereas later instars feed on all aboveground plant tissues. In well drained soils, 3rd instar larvae pupate in the soil or leaf litter underneath the host plant, whereas in flooded areas larvae pupate in the aerenchymous stem tissue produced by the plant in response to inundation. Development from egg to adult takes 30 to 40 d. In central New York, tenerals emerge in late June and feed and may reproduce for several weeks before overwintering (Blossey 1995b, a, Blossey and Schroeder 1995). Under favorable circumstances, a complete second generation may occur. *Galerucella* are the most abundant and widespread biocontrol agents of purple loosestrife. At sites where *Galerucella* reach high abundance they can defoliate entire purple loosestrife populations, reducing plant performance and biomass (Blossey 1995b, Blossey et al. 2001b).

Predator exclusion experiments

The experiments were conducted at eight sites in Central New York, USA between 2004 and 2006. All sites were heavily invaded by purple loosestrife and both species of *Galerucella* were released in 1999. In 2004, we tested the effects of flooding regime, predator exclusion and *Galerucella* initial density on *Galerucella* egg survival at two sites in the Northern Montezuma Wetlands Complex (NMWC). In 2005 we ran a similar test at one site in NMWC, but we also conducted separate assays for larval and pupal survival. Assays were run chronologically for each developmental stage: first egg, then larvae, and finally pupae because their order of appearance in the

field determines the identity and quantity of predators *Galerucella* are exposed to.

To assess if top-down forces reduce *Galerucella* larval survival and hence the success of the purple loosestrife biological control program, we experimentally excluded natural enemies in 2006 at four sites with lack of purple loosestrife control in Bridgeport NY and 15 miles away at four sites with successful control in NMWC (personal observation, B Blossey & V Nuzzo unpublished data). We chose sites in NMWC with a permanently flooded area adjacent to a non-flooded area allowing us to evaluate the effect of flooding regime on larval survival and biomass.

At each site, we randomly selected one experimental purple loosestrife stem per plant and protected it from herbivory with a gauze bag before *Galerucella* became active each spring. Following a factorial design, each stem was randomly assigned to the following treatments: two levels of flooding regime (permanently flooded, non-flooded), two levels of predation (exposed or not exposed) and three levels of *Galerucella* density (low, medium, high; only two levels in 2004), with 10 replicates per treatment combination in 2004-05 and 15 replicates in 2006.

To test the effect of flooding regime we selected sites at NMWC that had adjacent flooded and non-flooded areas and randomly chose half the stems in each habitat type. We manipulated predation exposure by removing gauze bags from half the stems (exposed to predation) in each area, while retaining gauze bags on the other half (protected from predation). Although gauze bags may change plant growth and microclimate, which may affect *Galerucella* survival and performance, the short difference in caging of plants

(3 d) in exposed and protected treatments is expected to be of little consequence to our results.

Initial *Galerucella* density was manipulated by placing the desired number of eggs, 1st or 3rd instar larvae onto each experimental stem. To establish *Galerucella* egg density, we released *Galerucella* adults onto each experimental stem and allowed them to oviposit for two days. In order to standardize where eggs were laid and also to facilitate egg recovery, we contained the beetles in the upper portion of each stem using a clip-on gauze bag. After removing adults, we removed excess eggs according to each density treatment, and marked egg position with a permanent marker on one side of each egg mass. To establish larval and pupal densities we transferred 1st and 3rd instar larvae, respectively, to each experimental stem according to the desired densities. We collected larvae from nearby infested purple loosestrife on the same day the assay started. *Galerucella* density treatments, low (5 eggs/stem or 1 larva/cm stem), medium (15 eggs/stem or 2 larva/cm stem), and high (30 eggs/stem or 4 larva/cm stem) represent a range of densities where we expect *Galerucella* to have no effect on purple loosestrife growth at the lower end, and where severe defoliation is expected at the higher level (Blossey 1995b, Landis et al. 2003). We did not distinguish between *G. calmariensis* and *G. pusilla* as it is impossible to differentiate eggs and larvae.

After three days, we collected and transported all stems to the laboratory to determine the proportion of predated eggs, live larvae and larval biomass (2006 only). Predated eggs have a flat appearance and they can be reliably distinguished from viable and hatched eggs under a stereomicroscope (Hunt-Joshi et al. 2005). For the pupal survival assay, we covered every

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experimental stem with a gauze bag immediately after placing the desired number of 3rd instar larvae onto each stem. When all larvae had crawled down to the soil or aerenchyma to pupate, we exposed half the stems to predation by removing gauze bags. After 3 d we covered all experimental stems with gauze bags and waited until adults emerged one week later, when we collected all stems and counted the number of emerged adults.

Plant quality experiment

To assess whether flooding regime influences purple loosestrife quality and has an effect on *Galerucella* performance, we conducted a common garden experiment in Richford, New York (42°23.22' N, 76°14.44' W) from 20 April to 2 July, 2006. We collected dormant purple loosestrife rootstocks on 4 April 2006 in Dryden, NY. Each rootstock was carefully washed to avoid destroying fine roots or the new shoots, weighed and then planted into a 28 cm diameter by 25 cm tall pot with pond soil. Soil was thoroughly mixed before placing it into each pot. Each pot was placed into a larger tree-pot (diameter 90 cm, 60 cm tall) lined with a plastic bag to retain water, and leveled with approximately 2 cm of sand.

Plants were randomly assigned to one of three flooding treatments: (1) dry (no standing water), (2) partially flooded (water depth 15 cm) and (3) completely flooded (water level at pot rim, approx. 25 cm). To achieve different flooding treatments, we perforated the plastic bags at different heights and filled tree pots with tap water. Plants in the dry treatment were watered only as needed to avoid wilting. Water levels were checked regularly and adjusted as needed. Pots were randomly arranged in six blocks with five replicates per block for a total of 15 replicates per treatment for plant and insect measures,

respectively. Plants in the first three blocks (n=45) were used to collect plant measurements, while plants in the last three blocks were used to collect insect measurements. Plant and insect measurements were collected on different experimental plants because herbivory may affect plant biomass and fitness, and may induce chemical responses (Karban and Baldwin 1997, Agrawal 1998).

Plant measurements: We measured seven plant traits on each experimental plant: biomass, height, leaf toughness, trichome density, C:N ratio, tannin concentration and water content. Final above- and below-ground biomass was determined after drying all plant material at 60°C for 72h. Before drying the samples, we thoroughly washed each plant and separated roots from stems and leaves. Height of the tallest stem for each plant was measured three times (3 May, 7 June and 2 July 2006). Leaf toughness was measured with a 50 g pesola spring scale (BioQuip®, Rancho Dominguez, CA) indicating the g of force necessary to penetrate a surface. Surface area was calculated using the diameter of the tip of the pesola. We selected the 4th leaf from the top and took two measurements per leaf: one on each side of the midrib. Both measurements were averaged to obtain a single data point per plant. Trichome density was estimated by counting trichomes on the top and bottom of one 28 mm^2 leaf disc per plant taken from the 3rd top leaf. On the bottom of the disc, we only counted trichomes along the midrib. C and N concentrations were measured from the 5th top leaf per plant. Leaf material was freeze-dried, ground and analyzed using a Carlo Erba NC2500 elemental analyzer at the Cornell Nutrient Analysis Laboratory. Water content was measured as the difference between fresh and dry weight of one 28 mm^2 leaf disc per plant. Leaf discs were taken from the tip of the 4th top leaf and were

kept on ice while in the field. Samples were weighed in the laboratory, dried at 60°C for 24h and then weighed again.

Tannin content was estimated from leaf extracts through a radial diffusion essay, which takes advantage of the ability of tannins to precipitate proteins (Hagerman 1987). We collected the top five fully expanded leaves from each experimental plant, then froze and ground the leaves. Plant tissue was extracted in 45% acetone solution, using 1 ml of solvent per 100 mg of dry plant weight. We placed 16 µl of plant extract in 4 mm wells punched into a Petri dish containing a mixture of agar and protein. We ran two replicates per plant sample and also a series of tannic acid standards. After placing the samples, Petri dishes were covered and incubated at 30°C for 96 h. Tannin in the samples react with the protein forming a visible ring. The square of the diameter of the ring is proportional to the tannin concentration in the extract. We took the average of two perpendicular measurements for each ring to minimize errors due to non-uniform ring development. A detailed protocol is available at Hagerman (1987, 2002). Tannic acid standards were used to relate the square of the ring diameter to the tannic acid concentration resulting in the following regression equation: $diameter^2 = -0.509 + 0.17$ tannic acid concentration (adjusted $r^2 = 0.996$, P =5.53e-6).

Insect measurements: We covered two stems per experimental plant with gauze sleeves and placed one mating pair of *Galerucella* onto each stem on 2 June 2006. After 24 h we removed one of the two stems per plant and counted the number of eggs laid under a stereomicroscope. We also removed adults and the gauze bag from the other stem and eliminated excess eggs leaving 15 eggs per stem. After 8 d we collected all stems, retrieved and counted all live larvae. Larvae were frozen, dried at 40°C for 3 d and weighed.

Statistical analyses:

Predator exclusion experiments: Experiments for each year and developmental stage were analyzed separately because the design changed slightly. For each experiment we determined the proportion of viable eggs, live larvae or adults recovered. For 2004 and 2005 data, we ran a three-way ANOVA to test the hypotheses that flooding regime, predation exposure and initial density affect *Galerucella* egg, larval or pupal survival. In 2006, to test the hypothesis that predation pressure is higher in regions with lack of purple loosestrife control vs. regions with successful control we compared the proportion of recovered *Galerucella* with a two way ANOVA. Region (NMWC or Bridgeport) and exposure to predation were included as fixed factors, and larval survival or biomass as response variables. We then examined each region independently using a mixed linear model with site as random factor, and exposure to predation and flooding regime (only at NMWC) as fixed factors. Test assumptions were examined and met for all cases. All tests were conducted in S-Plus 8.0 (Insightful Corporation 2005).

Plant quality experiment: We conducted a multivariate analysis of variance, MANOVA, with flooding treatment and block as the independent variables and the seven plant traits as response variables. Initial fresh rootstock biomass was included as a covariate in the analysis. We only used the final height measurement in the model. Initial biomass, toughness, trichome density and water content were log transformed to correct for lack of normality and reduce variance heterogeneity. We conducted a second MANOVA to test the effect of flooding treatment and block on the three *Galerucella* performance variables (oviposition rate, larval survival and larval biomass). Subsequent, two-way ANOVAs were performed to test for the

effect of flooding and block on each plant and insect measure. If the treatment effect was significant, each ANOVA was followed by pairwise comparisons using Tukey's multiple comparison test (α=0.05, except for leaf toughness α=0.0525). All tests were conducted in S-Plus 8.0 (Insightful Corporation 2005).

Results

Predator exclusion experiments

We observed a consistent significant effect of flooding regime and density on *Galerucella* survival; however, the effect of predation was in most cases not significant. Results were consistent among years, sites and developmental stages. In 2004, both sites at NMWC were analyzed independently because samples from the flooded area of one site were damaged. We did not find a significant effect of exposure to predation ($F_{1,72}$=1.21, P=0.275 for site A; $F_{1,72}$=0.20, P=0.655 for site B) or initial *Galerucella* density ($F_{1,72}$=0.60, P=0.442 for site A; $F_{1,72}$=0.75, P=0.393 for site B) on egg survival (Fig. 1.1). While egg survival significantly increased in the flooded area compared to the non-flooded area at site A ($F_{1,72}$=4.53, P=0.036) we found no interaction with exposure to predation ($F_{1,72}$=0.15, P=0.691) or density ($F_{1,72}$=0.75, P=0.389). On average 0.61 ± 0.05 (mean proportion surviving ± 2SE) proportion of viable eggs was retrieved from the flooded area vs. 0.46 ± 0.04 from the non-flooded area (Fig. 1.1).
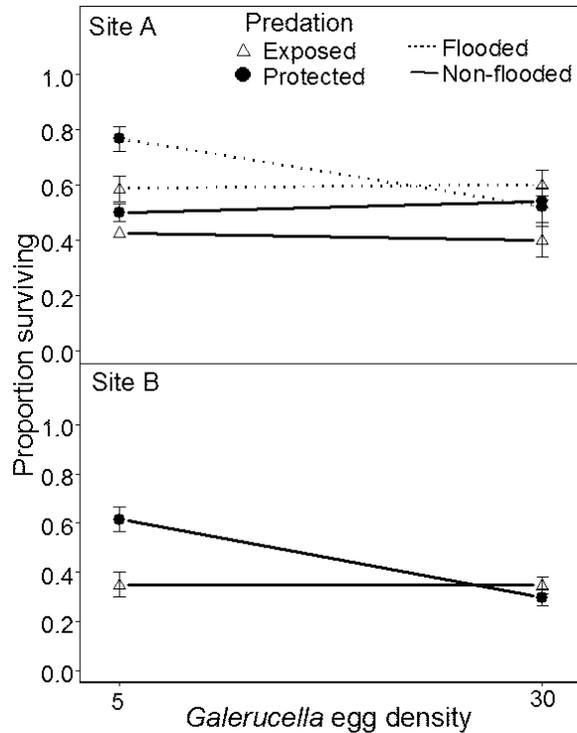
Figure 1.1. Effect of flooding regime, predation (exposed or protected) and initial *Galerucella* density on egg survival at two sites in the NMWC in 2004. Data are mean proportion surviving ± 2SE of 10 replicates/treatment.

In 2005, egg, larval and pupal survival were significantly higher in flooded areas compared to survival in non-flooded areas (Table 1.1, Fig. 1.2). Exposure to predation did not affect egg or pupal survival, but it significantly decreased larval survival (Table 1.1, Fig. 1.2). Larval and pupal survival decreased as initial *Galerucella* density increased, but egg survival was not affected (Table 1.1, Fig. 1.2). None of the interactions were significant (Table 1.1). However, when each habitat (flooded and non-flooded) was analyzed independently, predation significantly reduced larval survival only in non-flooded areas ($F_{1,54}=5.24$, P=0.026 for non-flooded; $F_{1,54}=0.19$, P=0.661 for

flooded), whereas density reduced larval survival only in flooded areas ($F_{1,54}$=0.88, P=0.419 for non-flooded; $F_{1,54}$=7.74, P=0.001 for flooded).

Table 1.1. Summary of ANOVA results for the effects of flooding regime, predation (exposed or protected) and initial *Galerucella* density on egg, larval and pupal survival at NMWC in 2005.

	df	Egg survival		Larval survival		Pupal survival	
		F	P	F	P	F	P
Flooding regime (F)	1,108	19.67	**2.2E-05**	25.38	**1.9E-06**	46.84	**4.8E-10**
Predation (P)	1,108	1.17	0.282	3.63	**0.059**	0.79	0.377
Density (D)	2,108	0.04	0.963	6.94	**0.001**	5.82	**0.004**
F * P	1,108	0.46	0.499	1.61	0.207	2.19	0.142
F * D	2,108	0.17	0.843	1.95	0.148	2.47	0.089
P * D	2,108	1.90	0.154	0.04	0.963	0.79	0.455
F * P * D	2,108	0.41	0.663	0.26	0.769	0.12	0.883
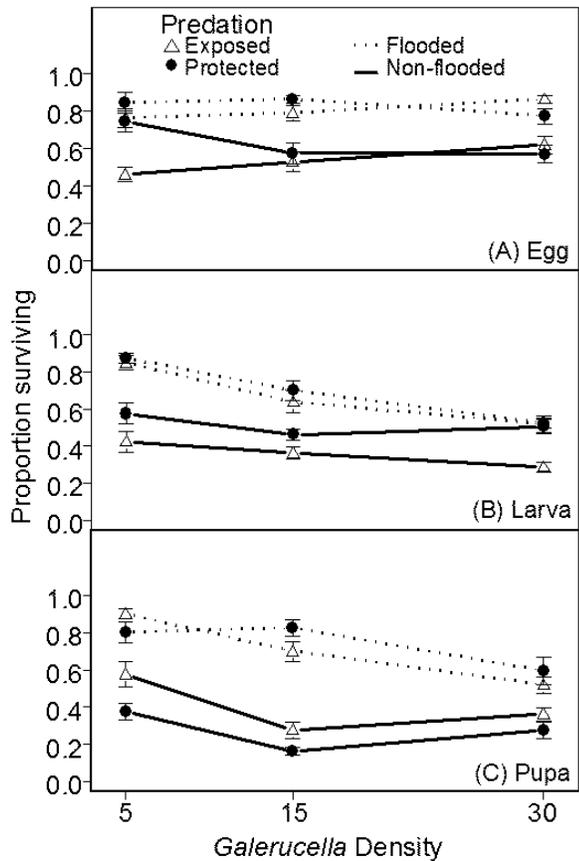
Figure 1.2. Effect of flooding regime, predation (exposed or protected) and initial *Galerucella* density on (a) egg, (b) larval, and (c) pupal survival at NMWC in 2005. Data are mean proportion surviving ± 2SE of 10 replicates/treatment.

In 2006, we found significantly higher larval survival ($F_{1,322}$=4.04, P=0.045) and larval biomass ($F_{1,322}$=44.68, P=9.82 x 10^{-11}) in the region with successful control (NMWC) compared to the region with lack of purple loosestrife control (Bridgeport). We did not find a significant effect of exposure to predation on larval survival ($F_{1,322}$=0.96, P=0.328) or larval biomass ($F_{1,322}$=0.28, P=0.596); nor did we find a significant region x predation interaction ($F_{1,322}$=0.10, P=0.754 for survival; $F_{1,322}$=0.2, P=0.652 for biomass)

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indicating no difference in the effect of predation between both regions (Fig.

1.3). *Galerucella* at NMWC had higher larval survival and larval biomass than

at Bridgeport even when we excluded the samples from the flooded area at

NMWC, and only compared non-flooded samples from both region

($F_{1,212}=7.54$, P=0.007 for larval survival; $F_{1,212}=13.54$, P=2.9×10^{-4} for larval

biomass).
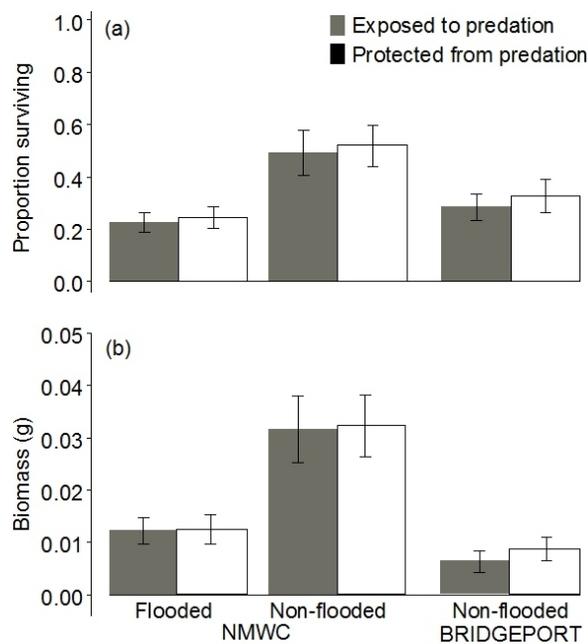
Figure 1.3. Effect of flooding regime and predation (exposed or protected) on

Galerucella (a) larval survival (proportion surviving) and (b) larval biomass (g)

at sites with successful purple loosestrife control (NMWC) and with lack of

control (Bridgeport). Data are means ± 2SE of 15 replicates/treatment at 4

sites per region.

When the two regions were analyzed independently, we found significantly higher larval survival ($F_{1,233}$=78.56, P=2.2e-16) and larval biomass ($F_{1,233}$=76.35, P=4.4e-16) in the flooded areas than in the non-flooded areas at all sites at NMWC. Exposure to predation had no significant effect on larval survival ($F_{1,233}$=0.56, P=0.456) or larval biomass ($F_{1,233}$=0.05, P=0.829) and it did not interact with flooding regime ($F_{1,233}$=0.03, P=0.87 for larval survival; $F_{1,233}$=0.01, P=0.926 for biomass, Fig. 1.3). At Bridgeport, predation did not affect larval survival ($F_{1,91}$=0.45, P=0.230) or larval biomass ($F_{1,91}$=2.47, P=0.119), except at one site (Site 4) adjacent to a permanently flooded area invaded by purple loosestrife. At this site exposure to predation had no effect on larval survival ($F_{1,22}$=0.48, P=0.50) but it significantly decreased larval biomass ($F_{1,22}$=8.10, P=0.009, Fig. 1.4).
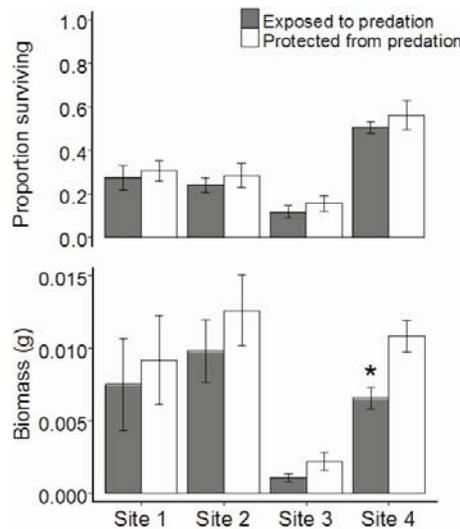
Figure 1.4. Effect of predation on (a) *Galerucella* larval survival (proportion surviving) and (b) biomass (g) at four sites in Bridgeport in 2006. Significant differences are indicated with *. Data are means ± 2SE of 15 replicates/treatment/site.

Plant quality

Flooding treatment and block had a significant effect on the seven plant traits measured (MANOVA, $F_{14,44}$=3.19, P=0.001 for flooding treatment; $F_{14,44}$=2.61, P=0.007 for block) but the flooding treatment x block interaction was not significant ($F_{28, 96}$=0.98, P=0.507). Initial rootstock biomass was highly significant ($F_{7,21}$=23.92, P=0) and therefore, included in the model as a covariate. Subsequent ANOVAs indicated that only leaf toughness, water content and tannin concentration were significantly affected by flooding treatment (Fig. 1.5). A posteriori pairwise comparison showed that leaf toughness of plants in the flooded treatment was 15% higher than in plants growing in the partially flooded treatment, but it did not differ from plants in the dry treatment. Plants in the flooded treatment had 55% lower tannin concentration than plants grown in the dry treatment, and 20% higher water content than plants grown under dry and partially flooded treatments. Block had a significant effect on leaf toughness ($F_{2,36}$=8.32, P=0.001) and tannin concentration ($F_{2,36}$=9.09, P=6.35e-4), but not on water content ($F_{2,36}$=1.46, P=0.24). In all cases the flooding treatment x block interaction was not significant ($F_{4,36}$=1.73, P=0.16 for leaf toughness; $F_{4,36}$=2.51, P=0.06 for tannin concentration; $F_{4,36}$=0.75, P=0.56 for water content). Results were consistent if block was not included in the model.
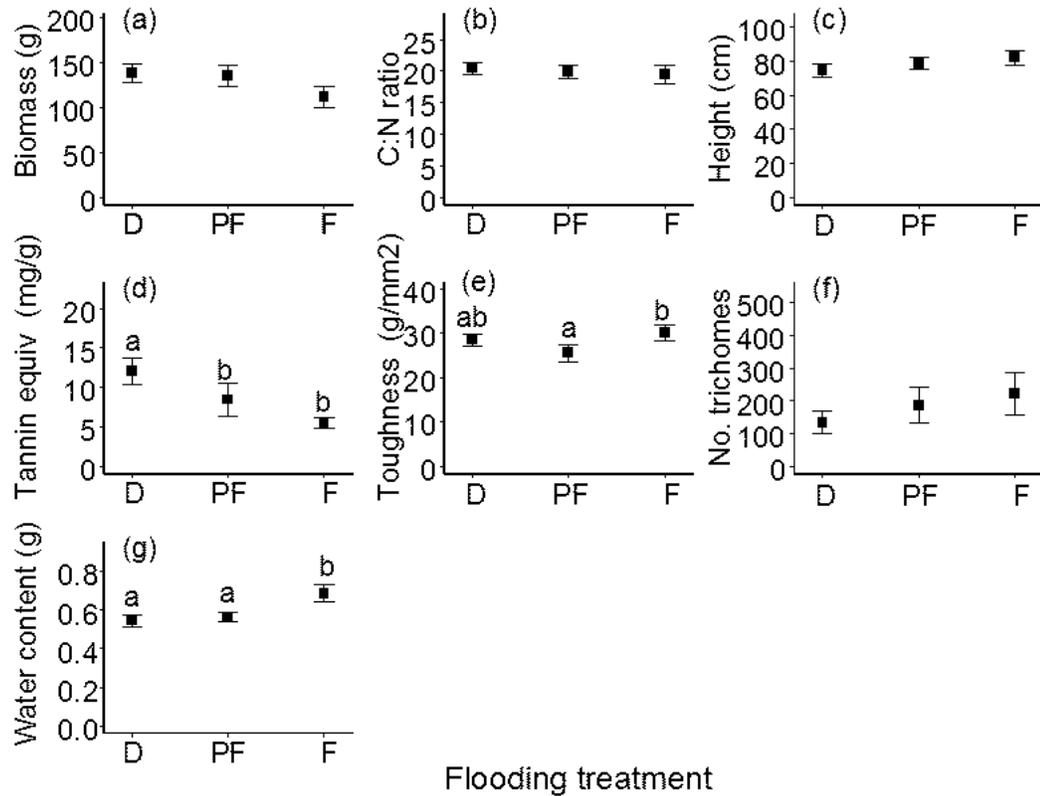
Figure 1.5. Effects of flooding treatment (D=dry, PF= partially flooded, F= completely flooded) on purple loosestrife traits: (a) total dry biomass (g), (b) C:N Ratio, (c) height (cm), (d) tannin content(mg/g), (e) leaf toughness (g/mm2), (f) trichome density, and (g) water content (g). Data are means ± 2SE of 15 replicates/treatment. Significant differences between means are indicated with different letters (Tukey's test, α=0.05, except for leaf toughness α=0.0525).

Galerucella performance was also significantly affected by flooding treatment (MANOVA, $F_{6,70}$=2.35, P=0.039), but in this case block had no effect ($F_{6,70}$=1.06, P=0.396) and it did not interact with flooding treatment ($F_{12,108}$=0.85, P=0.594). Subsequent ANOVAs indicated that oviposition rate and larval survival differed among flooding treatments. Galerucella laid 34%

more eggs on flooded plants compared to plants in the dry treatment, and larval survival was 40% higher in the partially flooded treatment compared to the dry treatment (Fig. 1.6).
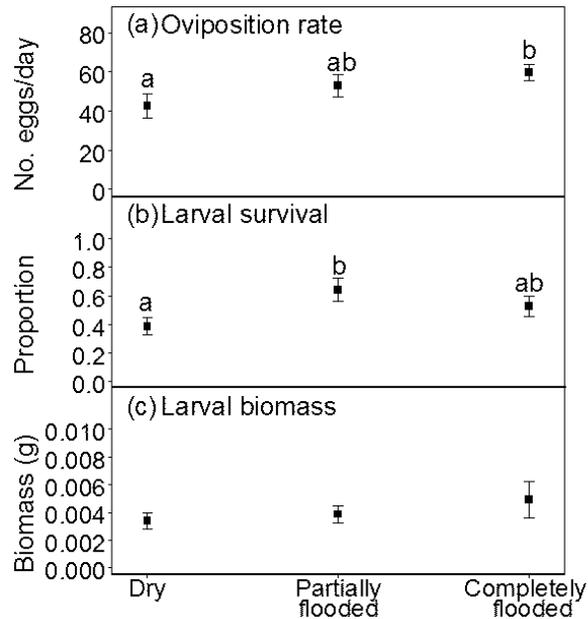
Figure 1.6. Effect of flooding treatment on *Galerucella* performance measures: (a) oviposition rate (# eggs/d), (b) larval survival (proportion surviving) and (c) larval biomass (g). Data are means ± 2SE of 15 replicates/treatment. Significant differences of the means are indicated with different letters (Tukey's test, α=0.05).

Discussion

Our results confirm that flooding benefits *Galerucella* leaf-beetles, but only partially support the hypothesis that flooded environments provide refuge from predation (Menge and Sutherland 1976). Flooding increased *Galerucella* survival in all occasions independent of exposure to predation. However, the effect of

predation at our sites was minor and highly localized: it only reduced survival of *Galerucella* larvae and did not affect egg or pupal survival, and its effect was significant only at two out of eight sites and only during one year of the study. Although predation was recognized as a possible limiting factor for *Galerucella* species at the onset of the biocontrol program (Malecki et al. 1993) and laboratory and field experiments have identified an ample suite of generalist predators that can prey on *Galerucella* (Sebolt and Landis 2004, Wiebe and Obrycki 2004, Hunt-Joshi et al. 2005) our results question the generality and importance of predation as a limiting factor at locations where *Galerucella* populations fail to control purple loosestrife. Our results support previous research reporting a limited effect of predation in the field (Sebolt and Landis 2004), although occasional strong predation effects are reported for certain years or sites. For example, Sebolt and Landis (2004) reported that adult emergence significantly decreased in un-caged purple loosestrife stems in one year at a single site indicating that predators were able to reduce the rate of population growth. Similarly, previous research conducted at one of our sites found a strong potential for the mirid bug, *Plagiognathus politis*, to affect *G. calmariensis* populations (Hunt-Joshi et al. 2005). However, long-term monitoring of the site has shown that predation did not prevent *Galerucella* outbreaks and successful purple loosestrife control (Blossey and Nuzzo, unpublished data).

Results we obtained in our region may not be applicable to all other areas where *Galerucella* has been released. For example, research in Iowa, USA indicates severe and widespread predation on *Galerucella* eggs and larvae (Wiebe and Obrycki 2004). Additionally, our results may underestimate the overall effect of predation on *Galerucella* populations because our exposure times were less than life stages would usually encounter during their

development in the field. We also did not evaluate predation as a factor of adult mortality during oviposition, dispersal or overwintering. Nevertheless, available evidence indicates that biotic interference is not as common as expected for *Galerucella* and that its strength varies spatially and temporally.

We expected flooding to decrease top-down control as it might limit dispersal of ground-dwelling predators. However, common *Galerucella* predators such as ladybird beetles (Coccinelidae), ground beetles (Carabidae) and spiders were collected in flooded areas at our sites during exploratory surveys (data not shown) conducted throughout the course of the experiment. Similarly, flooding did not constitute a barrier to predators of the closely related *Galerucella lineola* (Sipura et al. 2002). Although flooding did not provide an enemy free refuge, it might affect *Galerucella* survival through changes in adult density. Increased adult density in flooded areas may reduce the negative effects of predation, but increase intraspecific competition. However, our results show that even though *Galerucella* faced higher competition for purple loosestrife resources and pupation sites in flooded areas its survival was still higher than in non-flooded areas.

Predation is often cited as a reason why biocontrol agents fail to establish or to increase population size in their introduced range (Crawley 1989, Lawton 1990); however, there are few quantitative studies confirming anecdotal evidence of the importance of predation (Goeden and Louda 1976, Pratt et al. 2003). Our results show that predation pressure did not vary among sites with successful control of purple loosestrife compared to sites with lack of control, indicating that causes other than predation are responsible for lack of purple loosestrife control at these sites. In our study, poor purple loosestrife control occurred on drier sites, and except for one location, sites

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were not adjacent to flooded areas. Results of our flooding experiment
indicate that flooding can significantly increase *Galerucella* survival resulting in
better purple loosestrife control, improved recruitment and therefore, may
affect control at non-flooded sites through dispersal of adults. Flooded areas
adjacent to non-flooded areas can serve as donor sites from which *Galerucella*
disperse and colonize nearby drier sites. *Galerucella* forms aggregations
(Grevstad 1998) that may completely defoliate patches of purple loosestrife,
prompting long and medium-distance flights to less damaged resources
(Grevstad and Herzig 1997, Landis et al. 2003). Nearby drier sites with lower
Galerucella abundance and healthier purple loosestrife resources will be
colonized as resources in flooded sites get depleted. It is important to note,
however, that *Galerucella* survival at NMWC (successful control) was higher
than at Bridgeport (lack of control), even when only the dry areas from both
regions were compared. This indicates that flooding regime may not be the
sole factor responsible for lower *Galerucella* performance at dry sites in
Bridgeport.

Our results indicate that flooding likely offers a major benefit other than
providing refuge from predation to *Galerucella*. Purple loosestrife plants
grown in flooded soils did not have higher nutrient (C and N) concentration but
had significantly higher water content and lower tannin concentration and
tended to have lower leaf toughness, indicating that flooding may influence
purple loosestrife traits associated with nutritional quality. A positive effect of
flooding on host plant nutritional quality was also reported by Lower et al.
(2003) who found lower salicortin concentration in *Salix sericea* grown in
flooded low-N treatments and higher leaf protein in flooded high-N treatments
compared to non-flooded treatments. In addition, results are consistent with

other studies that have found higher phenolic concentration in plants grown in drier soils compared to flooded soils (English-Loeb et al. 1997), but opposite patterns also exist (Sipura et al. 2002).

Higher water content can improve digestibility of plant material and has frequently been associated with increased herbivore performance (English-Loeb et al. 1997, Scheirs and Bruyn 2005). Higher water content in purple loosestrife plants grown in flooded soils coupled with higher aerial moisture in flooded wetlands may reduce *Galerucella*'s risk of desiccation, which may be high for soft-bodied small organisms (Willmer 1982, Addo-Bediako et al. 2001) such as *Galerucella* larvae or eggs. Aerial moisture influences the distribution of the closely related *G. lineola* larvae (Sipura et al. 2002), which take advantage of host plant structures, such as leaf rolls, to protect itself from desiccation (Larsson et al. 1997). In addition, increased aerial moisture and temperature in flooded wetlands allow earlier purple loosestrife growth (personal obs.) providing a competitive advantage for purple loosestrife plants and likely affecting *Galerucella* host-selection as both species usually prefer taller, bigger plants (Blossey and Kamil 1996).

Lower tannin concentrations in purple loosestrife plants grown in flooded soils potentially increase nutritional plant quality for *Galerucella*. Tannins are considered general defensive agents (Feeny 1970) capable of decreasing herbivore fitness (Karowe 1989, Barbehenn and Martin 1994, Lill and Marquis 2000). However, tannins do not always have a detrimental effect and may even be beneficial (Bernays et al. 1980, Bernays and Woodhead 1982, Karowe 1989). This variation is likely due to the ample structural variation of tannins (Zucker 1983, Ayres et al. 1997) which produces different effects depending on species-specific plant-insect interactions (Bernays 1981,

Ayres et al. 1997, Forkner et al. 2004). For example, the leaf-beetle *Chrysomela falsa* Brown is sensitive to tannin extracts from hosts it rarely selects, but is highly tolerant to tannins from its preferred host *Populus tremuloides* Michx (Ayres et al. 1997). Given the reported variation in herbivore sensitivity to tannins, further research is required to establish if *Galerucella* is negatively affected by tannins contained in purple loosestrife plants before it can be determined whether higher *Galerucella* performance in flooded plants was due to higher host nutritional quality.

In conclusion, both bottom-up and top-down forces influenced *Galerucella* populations at our sites, but their relative strength was strongly affected by flooding. Flooding provided a double benefit to *Galerucella*: reduced predation and increased host plant quality, suggesting that top-down control decreases and bottom-up control increases as soils get flooded. Nevertheless, we suspect stronger bottom-up effects since top-down control was highly localized and *Galerucella* is known to avoid negative effects of predation through several predator avoidance mechanisms (Sebolt and Landis 2002, Hunt-Joshi et al. 2005). Local variations in plant ontogeny (*Galerucella* prefers younger tissues), predator subsidies from neighboring sites and herbivore composition would affect the strength of top-down and bottom-up forces and vary their impact along a flooding gradient. Under this heterogeneous milieu it is necessary to include landscape factors (Gripenberg and Roslin 2007) to fully understand how top-down and bottom-up forces influence *Galerucella* along environmental gradients. Our study shows that bottom-up and top-down control are not isolated forces, but are mediated by many environmental variables that should be integrated into studies of trophic interactions.

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CHAPTER 2

MATRIX HABITAT AND PLANT DAMAGE INFLUENCE COLONIZATION OF PURPLE LOOSESTRIFE PATCHES BY SPECIALIST LEAF-BEETLES

Abstract

The characteristics of the matrix habitat, i.e. the unsuitable habitat connecting host-plant patches, may facilitate or limit herbivore movement thus affecting their population dynamics and management. We evaluated the effect of matrix habitat, isolation and plant damage on movement of two leaf-beetles (*Galerucella calmariensis and G. pusilla*) introduced to North America as biocontrol agents of the invasive purple loosestrife (*Lythrum salicaria*). Mark-recapture/resight experiments indicated that leaf-beetles are more likely to colonize purple loosestrife patches embedded in meadow than in forest habitat; that previously attacked purple loosestrife plants are preferred over un-attacked plants, especially in the forest habitat; and that colonization rate decreased with distance. Movement of both species was similarly affected by matrix type, isolation and plant damage, but *G. pusilla* recaptures were always higher than *G. calmariensis* and did not vary amongst sites, whereas *G. calmariensis* did. Low colonization rates of purple loosestrife patches embedded in forests suggest that management practices should be tailored to redistribute leaf-beetles to areas surrounded by forests and that other management options should be implemented, as it is unlikely that *Galerucella* will to sustain outbreak populations at those sites.

Introduction

In natural landscapes, quality, density and distribution of suitable habitat typically vary across space (Hanski 1999). Spatial resource variability can profoundly affect herbivore dynamics, influencing colonization and extinction rates and local abundance (Robinson et al. 1992, Tilman and Kareiva 1997), as well as trophic (Fagan et al. 1999, Cronin 2003, Denno et al. 2005) and mutualistic (Diekötter et al. 2007) interactions. While the effects of fragment area, shape and isolation on herbivores have received much attention, the importance of the surrounding matrix and the associated edge effects have only recently been addressed.

A growing body of evidence indicates that herbivore dispersal abilities are affected by the surrounding matrix (Kareiva 1985, Pither and Taylor 1998, Roland et al. 2000, Jonsen et al. 2001, Ricketts 2001, Haynes and Cronin 2003, Hein et al. 2003, Matter et al. 2004, Haynes et al. 2007). For example, meadow-dwelling butterflies prefer to cross through willow than conifer matrix, although the effect is taxa-specific (Ricketts 2001). Similarly, the planthopper *Prokelisia crocea* (Van Duzee) would rather cross through patches of the exotic grass smooth brome (*Bromus inermis* Leyss) than through mudflats (Haynes and Cronin 2003). Consequently, physical location of a patch and the permeability of the surrounding matrix determine connectivity (Ricketts 2001). In sum, highly permeable matrices increase the effective connectivity among patches, while hard matrices reduce movement thereby increasing isolation.

The effect of matrix composition is key to understand herbivore dynamics in patchy environments and could be of great importance for biological invasions and biological control (Jonsen et al. 2001). Success of a

biological control program depends on the ability of the agent to establish and to reach outbreak populations that exert substantial damage to the target. In addition, biocontrol agents should be able to spread without human assistance, and their rate of dispersal likely influences the rate at which targets are controlled: low dispersal will decrease the agent's rate of spread and therefore the area under which the target is controlled (Fagan et al. 2002), whereas high dispersal may thin populations putting them at extinction risk from environmental and demographic stochasticity or an Allee effect (Grevstad 1999). In addition, variation in dispersal abilities of biocontrol agents may result in different control agent distributions. For example, the flea beetle *Aphthona nigriscutis* (Foudras) introduced to control leafy spurge (*Euphorbia esula* L.) has higher probability of immigrating into a leafy spurge patch when moving through a grass than a shrub matrix, whereas movement of another agent, *A. lacertosa* (Rosch) did not differ between matrices but dispersal was much lower overall than for *A. nigriscutis* (Jonsen et al. 2001). Despite the potential importance of landscape structure for biocontrol success, its effect has rarely been considered (but see Grevstad and Herzig 1997, Jonsen et al. 2001, Zaviezo et al. 2006).

Here we explore the interactive effects of landscape structure (matrix and isolation) and host-plant damage on movement of two leaf-beetles (*Galerucella calmariensis* L. and *G. pusilla* Duft.) introduced to North America to control the invasive weed purple loosestrife (*Lythrum salicaria* L.) (Malecki et al. 1993). *Galerucella* spp. are the most widespread of all control agents released against purple loosestrife, partly because they are easily mass-reared under greenhouse conditions (Blossey and Hunt 1999), but also because both species are strong fliers and able to colonize distant purple

loosestrife patches (Grevstad and Herzig 1997). Over a 10 y period *G. calmariensis* and *G. pusilla* released at six locations in Central New York State colonized at least 32 new *L. salicaria* patches (mean dispersal distance 3368 ± 534.95 m, Blossey unpublished data). Despite *Galerucella* spp. widespread distribution, at certain sites insects fail to increase population size to critical levels (Landis et al. 2003, Blossey and Nuzzo 2004, Grevstad 2006). Top-down control and flooding regime have been proposed as the main reasons for low *Galerucella* recruitment at drier sites (Landis et al. 2003, Blossey and Nuzzo 2004, Sebolt and Landis 2004). In addition, previous experiments indicate that *Galerucella* spp. rapidly disperse from purple loosestrife patches surrounded by forests (unpublished data) indicating that insect dispersal may play a role on the success of the biocontrol program.

Galerucella spp. are strongly attracted to conspecifics (Blossey 1995a, Grevstad and Herzig 1997) due to a male secreted pheromone that attracts both species indistinctively (Bartelt et al. 2006). As a result, adults form temporary aggregations that can cause localized defoliations (Grevstad and Herzig 1997, Landis et al. 2003). As plant resources are depleted, adults disperse with peak density occurring in moving "hot spots" across a patch (Landis et al. 2003). This strong aggregation pattern may increase purple loosestrife control because it produces heavy localized damage. However, it may also allow a rebound in *L. salicaria* as the plant is quickly released from chronic herbivore pressure when beetles disperse to less damaged plants. To reach new purple loosestrife patches, *Galerucella* spp. need to cross unsuitable habitat, including non-invaded marshes, shrubbery thickets, forests and water bodies. Therefore, *Galerucella* spp. dispersal ability through

unsuitable habitats is critical to guarantee successful control of purple loosestrife at the landscape level.

The main objective of this study was to examine the effect of matrix type (meadow and forest), isolation and herbivore damage on immigration of *Galerucella* spp. into purple loosestrife patches. We chose forest and meadow habitats because they are two highly contrasting matrices which represent the least and most similar habitat, respectively, to purple loosestrife invaded marshes. Using experimentally created purple loosestrife patches and a coupled mark-recapture and resight experiment we tested the hypothesis that *Galerucella* spp. are more likely to immigrate into patches surrounded by meadow than forest and to plants previously attacked by *Galerucella* spp. compared to un-attacked plants. In addition, we assessed how immigration differs between both species and sexes.

Methods

Study system: Purple loosestrife (*Lythrum salicaria*) is an invasive perennial wetland herb introduced to North America from Europe in the early 1800s (Thompson et al. 1987). Plants can grow across a wide moisture regime, but germination is limited to open moist soils where seedlings grow rapidly (> 1 cm/d). Mature plants can reach up to 2-3 m in height and develop 30-50 annual shoots that may produce 2.7 million seeds annually. Plants regrow each year from the woody rootstock which serves as a storage organ (Thompson et al. 1987, Mal et al. 1992a, Malecki et al. 1993, Blossey 1995a, b). Purple loosestrife can form nearly monospecific stands that may displace native vegetation and negatively affect amphibian and bird communities (Whitt

et al. 1999, Blossey et al. 2001b, Farnsworth and Ellis 2001, Brown et al. 2006).

Galerucella calmariensis and *G. pusilla* are specialist herbivores released in North America to control purple loosestrife. *Galerucella* spp. are sympatric species that share similar life histories (Blossey 1995a). Beetles overwinter as adults and emerge in spring. After a short feeding period, females mate and lay their eggs on purple loosestrife stems and leaves. Young larvae feed on the apical meristem and complete three instars before pupating in the soil or in the aerenchyma produced by purple loosestrife in response to inundation. Development from egg to adult takes 30 to 40 d (Blossey 1995b, a, Blossey and Schroeder 1995). In central New York, tenerals emerge in late June and feed and may reproduce for several weeks before overwintering. Under favorable circumstances, a complete second generation may occur.

Experimental design: The study was conducted at four sites near Ithaca, NY. Distance between sites ranged from 3 to 16 km and each one consisted of a meadow adjacent to a regenerating forest. The length of the border line between meadow and forest ranged from 100 to 200 m and each habitat (forest or meadow) extended for at least 125 m into opposite directions. Purple loosestrife and *Galerucella* spp. beetles were absent from all sites at the beginning of the experiment. To avoid staging an invasion, purple loosestrife plants were kept in pots which were removed at the end of the experiment before flowering and seed set had started.

At each site we created experimental purple loosestrife patches consisting of five potted plants each. We created artificial patches to decrease differences due to plant quality and patch shape and size (Haynes and Cronin

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2004). Experimental patches were located at 25, 50 and 100 m along transects radiating from the release point situated at the border between the forest and the meadow. Two transects extended into the meadow and two into the forest matrix. Purple loosestrife plants in one transect of each matrix type were previously attacked (25-50% leaf area removed) by *Galerucella* spp., whereas plants in the other transect were un-attacked (Fig. 2.1). Transects were randomly assigned to each herbivore attack treatment.
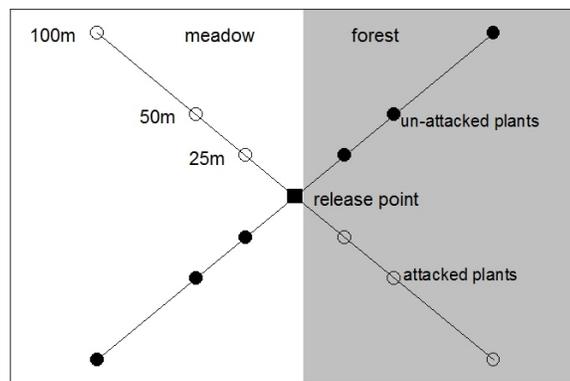
Figure 2.1. Schematic representation of the experimental landscape. Forest (gray), meadow (white), un-attacked plants (filled circles) and previously attacked plants (empty circles).

All purple loosestrife plants for the experiment where excavated as dormant rootstocks on 4 April 2006 in Dryden, NY. Each rootstock was carefully washed to avoid destroying fine roots and then planted into a 28 cm diameter by 25 cm tall pot with potting soil (Farfard Canadian growing mix No. 1-P, Agawam, Massachusetts, USA). Experimental plants were kept in a common garden under identical conditions until the start of the experiment. To impose the herbivore attack treatment (previous herbivore attack or no attack),

we covered all experimental plants (including controls) with a gauze bag and added 15 *Galerucella* spp. individuals to half the plants. After attaining the desired damage level (25 to 50% leaf area removed) we removed beetles and gauze bags from all plants. Plants were immediately transported to each experimental site and randomly positioned in experimental patches.

All beetles for the release experiments were collected in the Northern Montezuma Wetland Complex (Savannah, NY). Beetles were transported to the laboratory, chilled and marked using fluorescent powder (BioQuip®, Rancho Dominguez, CA, USA). After marking, beetles were individually aspirated and counted making sure that they were correctly marked and active (Kareiva 1982). A portion of the insects were kept in the common garden for the length of the experiment to check fate of marks. By the end of the experiment only 0.05% of beetles lost their marks and there was no difference in beetle survival between marked and unmarked individuals (t=0.92; P=0.361). Likewise, *G. calmariensis* was successfully marked with fluorescent powder in previous research and no effect on survival or movement was found (Grevstad and Herzig 1997). All insects were released within 24 h of collection.

We released 1200 marked beetles at each site on 30 May 2006. All releases were conducted after dusk to reduce beetle dispersal and activity. Releases at all sites were done within an hour to minimize differences in quality of the beetles at release time. Beetles were left at the release location at the border of both matrix types (meadow and forest) in an open 250 ml container filled with fresh purple loosestrife stems. *Galerucella* spp. show strong tendency to aggregate (Grevstad and Herzig 1997); therefore, we

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anticipated that beetles would disperse slowly, as purple loosestrife resources become depleted.

To assess the rate of colonization from the release point to each one of our experimental patches, beetles were both trapped and counted from 31 May to 10 June 2006. The number of *Galerucella* spp. on each purple loosestrife patch was counted for 1 min per patch daily from 800 to 1000 h. Sites were visited randomly to avoid trends due to potential differences in beetle activity during the day. We did not differentiate between *G. calmariensis* and *G. pusilla* in the field as species are indistinguishable unless dissected.

Galerucella spp. were also captured using paired sticky traps (15-cm^2 yellow sticky cards with adhesive on both sides, Olson Products, Medina, OH, USA). One trap was baited with a pheromone lure consisting of a red rubber septa loaded with *Galerucella* spp. pheromone, while the control trap had no pheromone lure (Bartelt et al. 2006). Pheromone lures were prepared by Dr. Bartelt, USDA Agricultural Research Service (Peoria IL, USA), shipped on 1 May 2006, and stored at our facility in Ithaca, NY at -15°C until the start of the experiment. Paired traps were located 3 m apart from each other on both sides of each experimental purple loosestrife patch. Each trap was suspended from a 1.30 m pole and the pheromone lure was attached to the trap top using a paper clip. All controls (un-baited traps) were set first to avoid contamination with pheromone. Lures were not replaced during the experiment due to limited availability. Sticky traps were replaced every other day and taken back to the laboratory where *Galerucella* spp. were carefully removed, checked for marking, identified and sexed. Beetles observed on purple loosestrife plants were not checked for marking, as the requisite

handling might have altered their behavior. However, sites had no previous populations of *Galerucella* spp. and all beetles captured on sticky traps were marked indicating that no beetles had immigrated into our sites.

Statistical Analyses: Count and trap data were analyzed separately using quasi-likelihood models with Poisson and binomial errors, respectively. We used quasi -likelihood models because our data was over-dispersed. Quasi-likelihood models estimate the dispersion parameter from the data, and therefore, produce more reliable significance levels when data is under- or over-dispersed (McCullagh and Neldar 1989). We first evaluated the effect of matrix type (meadow, forest), herbivore attack (previous attack or not), distance from release point (25, 50, 100 m) and site on the number of observed *Galerucella* spp. on purple loosestrife patches. To avoid the effect of time on recapture dates, we pooled data from the entire sampling period.

We fitted a second model for the sticky trap data, which evaluated the effect of species (*G. calmariensis* or *G. pusilla*) on the proportion of captured *Galerucella* spp. In this case, we pooled over the sampling period and trap type (baited traps with pheromone and un-baited traps). Pooling the data over the sampling period eliminated the effect of time and of changes in pheromone trap efficacy. Pheromone lures were not changed during the experiment; therefore, we expected pheromone trap efficacy to decrease over time due to decay. Un-baited sticky traps captured significantly less individuals than baited traps (F=58.49; P<0.001), but trap type did not interact with matrix type (F=0.29, P=0.59), herbivore attack (F=0.015; P=0.90) or distance (F=0.48, P=0.49); therefore, pooling data of both trap types increased the recaptured rates without altering the effects of the main factors on *Galerucella* spp. recapture rate.

Finally, we conducted separate analyses for each species, adding sex as an additional predictor. Due to the high number of insects that we needed for the release experiments, it was impossible to identify and sex all of them prior to release. A sub-sample of 420 insects were identified and sexed (130 male *G. calmariensis*, 78 female *G. calmariensis*, 120 male *G. pusilla* and 92 female *G. pusilla*) and these ratios were applied to the total number of insects released per site (n=1200) to estimate the number of individuals of each species and sex released at each site. All models were fit using S-PLUS 8.0 software (Insightful Corporation 2007).

Results

Mean recapture rates were low (4.57%), did not substantially vary among sites (3.71 – 5.5%) but differed between collection methods, with a 10-fold higher proportion of individuals observed on plants (0.124 ± 0.008) than captured with sticky traps (0.012 ± 5.4E-4 for pheromone-baited traps, 0.0004 ± 9.28E-5 for un-baited traps). Overall, a higher number of *Galerucella* spp. colonized purple loosestrife patches surrounded by meadow than forest, and their relative abundance decreased as distance from the release point increased (Table 2.1, Fig. 2.2). *Galerucella* spp. counts were significantly lower on un-attacked plants compared to previously attacked plants; however, its effect interacted with matrix type, with previous attack having no effect in meadows but significantly increasing *Galerucella* spp. counts in forests (Table 2.1, Fig. 2.2). Although the effect of each main factor (matrix, herbivore attack, distance) varied among sites (Table 2.1), the direction of the effect was consistent on all sites.

Table 2.1. Analysis of deviance table showing effects of site, matrix, herbivore attack and distance from release on the number of observed *Galerucella* in each purple loosestrife patch. The model is a Quasi-likelihood model fit with Poisson errors. A full model (main effects plus all interactions) was fit but only two-way interactions are presented as higher terms were not significant. Significant terms are in bold.

Term	df	Deviance	F-value	P(F)
Null	47	3991.26		
Site	3	193.92	10.69	**<0.001**
Matrix	1	1783.98	294.93	**<0.001**
Herbivore attack (HA)	1	80.22	13.26	**0.002**
Distance	1	325.94	53.88	**<0.001**
Site:Matrix	3	129.28	7.12	**0.003**
Site:HA	3	640.62	35.30	**<0.001**
Site:Distance	3	84.14	4.64	**0.016**
Matrix:HA	1	388.75	64.27	**<0.001**
Matrix:Distance	1	1.95	0.32	0.578
HA:Distance	1	23.26	3.85	0.068
Residual	16	97.14		
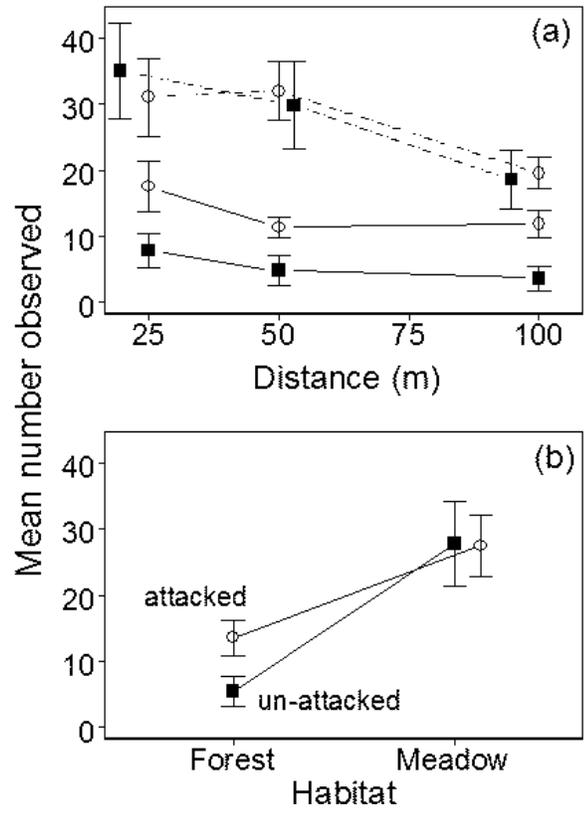
Figure 2.2. (a) Mean number of *Galerucella* observed in meadow (dashed line) and forest matrices (continuous line) according to distance and herbivore attack treatment (previous attack: open circles, no attack: filled squares). (b) Interaction plot of mean number of *Galerucella* observed in meadow and forest matrices according to purple loosestrife attack treatment. Error bars represent ± 2SE, n=4 sites.

Similar to count data the sticky trap results indicated that *Galerucella* spp. preferred meadow over forest matrix; however, site, previous *Galerucella* attack and distance had no effect on *Galerucella* spp. recapture rate, nor did we find a significant interaction among the main factors (Table 2.2). Both *Galerucella* species preferably colonized patches in the meadow compared to patches in the forest, but *G. pusilla* recaptures were significantly higher than

G. calmariensis (Fig. 2.3). Recapture rates of both species were similarly affected by distance and previous attack (Table 2.2, non significant species x distance interaction, F= 0.85, P=0.359 or species x herbivore attack interaction, F=0.06, P=0.803).

Table 2.2. Analysis of deviance table showing effects of site, matrix, herbivore attack, distance from release and species on the proportion of *Galerucella* captured with sticky traps. The model is a Quasi-likelihood model fitted with binomial errors. All second term interactions were fitted, but for clarity only main terms are shown as all interaction terms were not significant. Significant terms are in bold.

Term	df	Deviance	F-value	P(F)
Null	95	1432.05		
Site	3	78.53	1.66	0.183
Matrix	1	593.27	37.63	**<0.001**
Herbivore attack	1	1.94	0.12	0.726
Distance	1	34.50	2.19	0.144
Species	1	204.82	12.99	**<0.001**
Residuals	70	144.45		
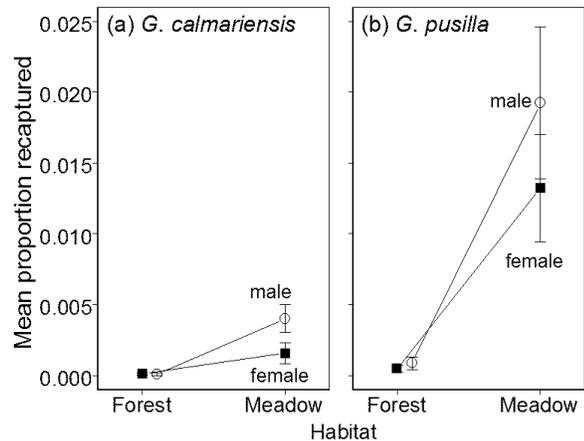
Figure 2.3. Interaction plot of mean proportion of (a) *G. calmariensis* and (b) *G. pusilla* recaptured in meadow and forest matrices according to sex. Error bars represent ± 2SE, n=4 sites.

Subsequent analyses for each species revealed that *G. calmariensis* recapture rate varied among sites whereas *G. pusilla* recapture rate did not (Table 2.3). Although overall previous herbivore attack had no effect on either species, *G. calmariensis* recaptures were higher on previously attacked plants at all but one site (significant site x herbivore attack interaction, Table 2.3). Similarly, distance had no effect on *G. calmariensis* or *G. pusilla* recaptures, but *G. calmariensis* recapture rate at each distance was affected by site (significant site x distance interaction, Table 2.3). For both species, recapture rates tended to be higher for males, but differences were not significant (Fig. 2.3, Table 2.3). Sex did not interact with site, matrix, previous *Galerucella* attack or distance for either species (Table 2.3).

Table 2.3. Effects of site, matrix, herbivore attack, distance from release, and sex on the proportion of *G. pusilla* and *G. calmariensis* captured in sticky traps. A separate Quasi-likelihood model with binomial errors was fitted for each species. All second term interactions were fitted, but for clarity only significant interactions and terms including the sex effect are shown. Significant terms are in bold.

Term	*G. pusilla*		*G. calmariensis*	
	F-value	P(F)	F-value	P(F)
Site	0.55	0.647	5.93	**0.001**
Matrix	11.35	**<0.001**	57.22	**<0.001**
Herbivore attack (HA)	0.08	0.781	0.68	0.412
Distance	1.04	0.312	1.13	0.291
Sex	0.19	0.663	0.00	0.981
Site: Matrix	0.10	0.960	0.73	0.540
Site:HA	0.65	0.588	3.67	**0.016**
Site:Distance	1.46	0.233	4.63	**0.005**
Site:Sex	0.01	0.998	1.61	0.196
Matrix:Sex	0.01	0.931	0.56	0.456
HA:Sex	0.06	0.810	1.26	0.266
Distance:Sex	0.01	0.930	0.03	0.869

Discussion

Our results clearly indicate that matrix habitat had a strong effect on the colonization rate of purple loosestrife patches by *Galerucella* spp. On average, *Galerucella* spp. were 2.9 times more likely to immigrate into purple

loosestrife patches embedded in meadow than forest habitats (Fig. 2.2).

Forest and meadow habitats have no value as a foraging resource for
Galerucella spp., since both are highly specific herbivores of purple loosestrife
(Blossey 1995a). Therefore, differential movement through either matrix is
likely related to matrix structural complexity, matrix permeability or to
Galerucella's avoidance behavior at the edge (Bowler and Benton 2005).
Higher permeability of the meadow matrix was expected because meadows
are a preferred habitat over forests for purple loosestrife (Mal et al. 1992b), are
structurally simpler than forests and are structurally similar to purple loosestrife
patches. Increased permeability of less structurally complex habitats (Jonsen
et al. 2001, Ricketts 2001, Matter et al. 2004) and of similar habitats (Kareiva
1985, Collinge and Palmer 2002, Haynes and Cronin 2004) has been reported
for several herbivorous insects. For example, migration of the butterfly
Parnassius smintheus Doubleday is reduced to a greater degree in more
complex forest habitats than in meadows (Matter et al. 2004), and cactus bugs
(*Chelinidea vittiger* Uhler) are less likely to emigrate from patches embedded
in taller more complex vegetation than from patches surrounded by shorter
vegetation (Schooley and Wiens 2004). Meanwhile, the prairie planthopper
(*P. crocea*) is more likely to cross through matrices that are structurally similar
to patches of its host plant (cordgrass, *Spartina pectinata* Bosc) than through
matrices that are structurally simpler (as mudflat) (Haynes and Cronin 2004,
Haynes et al. 2007).

Mark-recapture/resight experiments do not provide a mechanistic
understanding of the observed movement patterns. Nonetheless, we suspect
that differential colonization of purple loosestrife patches in forest and meadow
matrices is likely related to host-finding interference. *Galerucella* is less likely

to find its host when it grows inside shrubby thickets than outside. This effect is apparent when artificial shrubs are used indicating that visual cues are important during the host-finding process (Hambäck et al. 2003). Compared to meadows, forests are darker and visibility is considerably reduced likely affecting *Galerucella*'s host-finding ability. Complementary behavioral and small scale studies of *Galerucella*'s movement are needed to identify the mechanism underlying our results. Nevertheless, independent of the mechanism involved *Galerucella*'s ability to cross through or over forests is important for the species persistence at the landscape level and for the success of the biological control program. Purple loosestrife cannot grow in mature forests, but it can colonize open forest gaps in moist habitats, and forests patches frequently separate loosestrife patches, at least in the New York region (personal obs.).

Our data indicate that matrix habitat has a stronger effect than distance on *Galerucella* spp. movement, as matrix accounted for 44.7% of deviance, whereas isolation only explained 8.17% (Table 2.1). In addition, the effect of distance was significant only for resighted *Galerucella* and not for individuals trapped in sticky traps (Table 2.2). Although we did not find a significant interaction between habitat and distance, colonization of purple loosestrife patches embedded in forests was extremely low, and decreased only slightly with distance, even at the patches close to the edge (25 m). Evidence from other systems indicates an opposite pattern, where isolation and patch size were similarly (Haynes and Cronin 2003) or more important (Kareiva 1985, Moilanen and Hanski 1998, Goodwing and Fahrig 2002) than matrix habitat for insect movement. The observed differences may be related to experimental scale. Our experiment was conducted at relatively small distances (not at the

fine scale in Kareiva 1985, or at the landscape level in Moilanen and Hanski 1998) and we suspect that the influence of isolation increases at larger distances, as long-distance dispersal is associated with higher mortality (Grevstad and Herzig 1997). Despite these different trends, our data support increasing evidence that connectivity does not solely depend on distance among patches, but also on matrix characteristics and herbivore movement patterns (Ricketts 2001).

Previous herbivore attack significantly increased *Galerucella* immigration, especially to purple loosestrife patches embedded in forests, indicating that *Galerucella* is less likely to colonize new patches compared to patches that already have or recently had a *Galerucella* population. Previously damaged plants may indicate that host plant resources are suitable, which should be of especial importance in suboptimal habitats, such as in purple loosestrife patches surrounded by forests. Overall results suggest that when resources are plentiful *Galerucella* spread will be low, despite its inherent high dispersal ability (Grevstad and Herzig 1997). Therefore, control of purple loosestrife might be increased by actively redistributing *Galerucella* to new patches.

G. calmariensis and *G. pusilla* share the same ecological niche, and similar morphological and behavioral characteristics (Blossey 1995a); yet our results indicate that both species might have different dispersal abilities. At our sites, *G. pusilla* was more likely to immigrate into purple loosestrife patches than *G. calmariensis*, suggesting that *G. pusilla* is more likely to colonize nearer patches, while *G. calmariensis* is more prone to engage in long-distance dispersal. These results support previous observations that show that *G. calmariensis* disperses further than *G. pusilla* (Dech and Nosko

2002), but, at least at the scale of our experiment, contrast with results indicating that *G. calmariensis* is more likely to establish than *G. pusilla* (Dech and Nosko 2002). Given than long-distance flights are associated with increased mortality (Grevstad and Herzig 1997), *G. calmariensis* increased likelihood to engage in long-distance dispersal may reduce establishment rates for this species. Nevertheless, a ten year evaluation of the purple loosestrife biocontrol program in New York State, US showed that while *G. pusilla* was more likely to establish and was more abundant than *G. calmariensis* at initial release sites, but both species colonized equal number of new sites, likely indicating similar dispersal abilities (Grevstad 2006). Although both species are generally considered similarly important for long term control of purple loosestrife, differences in dispersal may render one species to be more important at a certain phase of control or a better control agent overall. Further experimentation is necessary to clarify potential differences in dispersal ability between both species and to determine whether these differences generate different spatial distributions for each species. For example, if *G. pusilla* prefers to colonize closer patches then it should be more common in sites located closer to the initial release location, whereas *G. calmariensis* should be more widespread.

Purple loosestrife is a highly invasive weed capable of forming large monospecific stands. Therefore, at early stages of control when purple loosestrife is abundant and relatively unfragmented, *Galerucella*'s ability to disperse through different matrix habitats should be of little importance, but it should become more important as purple loosestrife density and patch size decrease. At smaller and less dense patches purple loosestrife could be considered a transient resource, as *Galerucella* aggregation behavior may

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produce severe and localized defoliation, leading to complete depletion of purple loosestrife resources. Mobility then would become of great importance to guarantee *Galerucella*'s persistence in the landscape, as has been shown for other organisms that depend on transient resources (Denno et al. 1996, Bowler and Benton 2005).

Biological control science can greatly benefit from a more detailed understanding of the interactions among biocontrol targets and agents and landscape features (for example patch size and isolation, and matrix characteristics). The frequency and size of releases have long been a subject of interest among biocontrol scientist and practitioners (Grevstad 1999, Shea and Possingham 2000); however, our results demonstrate that landscape heterogeneity and agent dispersal ability should also be considered when planning release strategies. Specifically for purple loosestrife, results indicate that although *Galerucella* spp. can disperse over long distances (Grevstad and Herzig 1997), certain sites are unlikely to be colonized. Low colonization rates of purple loosestrife patches embedded in forests may reflect *Galerucella*'s limited ability to colonize forest patches indicating that management practices should be either tailored to redistribute leaf-beetles to these areas. Alternatively, if low colonization rates reflect *Galerucella's* active avoidance of purple loosestrife patches embedded in forests additional management options should be implemented, as it is unlikely that *Galerucella* will to sustain outbreak populations at those sites. In addition, maintenance and creation of meadow and marsh corridors will increase connectivity among purple loosestrife patches improving control. Understanding the effects of landscape features on biocontrol agent distribution can provide valuable insights for

management at a broader-scale and help predict and evaluate the extent of control (Jonsen et al. 2007).

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CHAPTER 3

EFFECTS OF HOST-PLANT DAMAGE AND DISTRIBUTION ON LEAF-BEETLE DISPERSAL

Abstract

Interactions between conspecific presence and host-plant damage may affect resource availability and hence influence an organism's dispersal behavior. We used a mark-recapture study and a spatially-explicit simulation model to evaluate the effect of host-plant damage and distribution, and presence of conspecifics on the dispersal behavior of two leaf-beetles (*Galerucella calmariensis and G. pusilla*) introduced to North America as biocontrol agents of purple loosestrife (*Lythrum salicaria*). The mark-recapture study revealed that leaf-beetles preferred purple loosestrife plants that had been previously attacked by conspecifics. Simple diffusion characterized movement over the whole plot, but not movement over sections with different levels of plant damage (no, low and high). Mean dispersal distance was higher in the no- and high plant damage sections of the plots compared to the medium damage section. The dispersal kernel had a fat tail in the high plant damage sections and a thin tail in the no- and medium damage sections. A factorial simulation experiment with degree of host-plant aggregation and insect density as factors revealed that mean dispersal distances were lower in the least aggregated landscape, but were not affected by insect density. Dispersal kernels for all cases were fat tailed and kurtosis increased with number of insects released and was higher for the least aggregated landscape. Results suggest that *Galerucella*'s dispersal behavior is affected

by landscape characteristics and conspecific presence, indicating that *Galerucella* dispersal can significantly affect purple loosestrife control.

Introduction

Habitat characteristics such as host availability (Kareiva and Odell 1987, Dwyer and Morris 2006, Chapman et al. 2007) and conspecific density ((Enfjäll and Leimar 2005, Rhainds et al. 2005), as well as individual characteristics such as sex and developmental stage (Markow and Castrezana 2000, Rhainds et al. 2002) may influence an individual's movement rate and whether it engages in long or short-distance dispersal. Resource-dependent dispersal that helps organisms cope with changes in resource abundance and distribution is common among insect species (Sutherland et al. 2002). Typically, dispersal is more likely when resources are scarce (Dwyer and Morris 2006), but when resources are plentiful or clumped insects tend to move less and have more tortuous paths (Kareiva and Odell 1987, Markow and Castrezana 2000). In addition, resource-dependent dispersal is of special importance for organisms depending on ephemeral resources or resources that become rapidly depleted by chronic herbivory (Denno et al. 1996).

Insects may use different cues to assess the abundance and quality of resources, including presence of conspecifics and host plant condition. Evidence indicates that some species disperse at a higher rate from low-density areas (Rouquette and Thompson 2007), whereas others will disperse at higher rates from areas with high conspecific density (Herzig 1995, Berggren 2005). Species that aggregate respond positively to conspecific density; however, as resources become depleted a migratory threshold should

be reached triggering local and long-distance dispersal. Thus, conspecific density may influence host condition (for example, damage level) and the effect of both factors on dispersal is likely dynamic and interdependent. In addition, responses to either factor may differ among and within species. For example, females of the goldenrod beetle *Trirhabda virgata* LeConte prefer plants with low herbivory but presence or absence of conspecifics does not affect their preference. Males, on the other hand, are attracted to conspecifics but show no preference among plants with different levels of defoliation (Herzig and Root 1996).

Here we explore the effects of conspecific density and host plant damage and distribution on the dispersal capability of two specialist leaf-beetles (*Galerucella calmariensis* L. and *G. pusilla* Duft., hereafter referred to as *Galerucella*) introduced to North America as biocontrol agents of the invasive weed purple loosestrife (*Lythrum salicaria* L.) (Malecki et al. 1993). *Galerucella*, which are now widely distributed in North America, have a strong dispersal capability reaching purple loosestrife patches almost one km away (Grevstad and Herzig 1997). Individuals are attracted to conspecifics (Blossey 1995a, Grevstad and Herzig 1997) due to a male secreted pheromone which attracts both species indistinctively (Bartelt et al. 2006). As a result, adults form temporary aggregations that can cause localized defoliation (Grevstad and Herzig 1997, Landis et al. 2003), but as plant resources are depleted, adults disperse with peak density occurring in a moving "wave" across a patch (Landis et al. 2003).

Understanding the dispersal capability of an organism is not only important to understand its dynamics, but also is critical to develop and evaluate management strategies (Turchin and Thoeny 1993, Liebhold and

Tobin 2008). For biological control the agent should establish sufficiently large populations to exert sustained damage on the target. However, the spread rate of the agent and its dispersal behavior may influence its efficacy, especially as the target species is controlled and available host patches become scarce (Fagan et al. 2002). Thus, the dispersal capability of *Galerucella* may affect the success of the biological control program. For example, low dispersal within a patch may create localized areas of control, while other areas are largely unaffected. Additionally, *Galerucella*'s tendency to aggregate may decrease the spread rate of these biocontrol agents, retarding purple loosestrife control at the landscape level.

In an effort to understand the mechanisms behind *Galerucella* dispersal and better predict its spatial distribution and dynamics we used a combination of mark-resight and simulation experiments to explore how conspecific presence, host plant damage and host-plant distribution affect *Galerucella*'s dispersal. A mark-resight experiment was conducted in small artificial landscapes composed of potted purple loosestrife plants that varied in the level of attack by *Galerucella* adults. To evaluate additional components of *Galerucella*'s behavior we then developed spatially explicit stochastic simulations where we varied conspecific density and purple loosestrife distribution.

Methods

Study system: Purple loosestrife (*Lythrum salicaria* L.) is a perennial wetland herb that can reach 2-3 m in height and develop 30-50 annual shoots. A single purple loosestrife plant may produce 2.7 million seeds annually, leading to the formation of a large seedbank (Welling and Becker 1990).

Germination is limited to open moist soils where seedlings grow rapidly (> 1 cm/d), but plants can grow across a wide moisture regime (Thompson et al. 1987, Mal et al. 1992a, Malecki et al. 1993, Blossey 1995a, b). Purple loosestrife is native to Europe, but highly invasive in North America where it can form nearly monospecific stands that may displace native vegetation and negatively affect amphibian and bird communities (Whitt et al. 1999, Blossey et al. 2001b, Farnsworth and Ellis 2001, Brown et al. 2006).

Galerucella calmariensis and G. pusilla are specialist purple loosestrife herbivores that share the same ecological niche (Blossey 1995a). Overwintering adults emerge in spring and feed for a brief period before reproducing, reaching peak oviposition in mid-June. First instar larvae feed on apical meristems, whereas older larvae and adults may feed on purple loosestrife leaves or stems. Larvae complete three instars before pupating in the soil or inside the aerenchyma produced by the plant in response to inundation (Blossey 1995b, a, Blossey and Schroeder 1995). In central New York, development from egg to adult takes 30 to 40 d and a complete second generation may occur under favorable circumstances. Both species were widely released in North America as purple loosestrife biocontrol agents in the 1990's (Hight et al. 1995, Blossey et al. 2001a).

Field Experiment: The study was conducted at three sites in Ithaca, NY, USA separated by 4 to 11 km. Each site consisted of a mowed area of at least 0.01 km^2 surrounded by meadows and shrubby vegetation. We used mowed areas to increase insect recovery and decrease effects of matrix habitat on Galerucella movement. Purple loosestrife and Galerucella beetles were absent from all sites at the beginning of the experiment. At each site, we

placed 90 similar sized potted purple loosestrife plants at 1-m intervals in an 8 x 9 m grid. Plants in each grid were arranged according to plant damage treatment: plants with medium damage level were placed in the three center columns of the grid and non- and highly damaged plants were placed on the three columns to the left and right side, respectively. All pots and plants were removed at the end of the experiment and before seed set to prevent spread of an invasive plant.

Purple loosestrife roots were collected at the Northern Montezuma Wetland Complex (Savannah, New York) on 23 May 2005. Each rootstock was planted into a 28 cm diameter by 25 cm tall pot with potting soil (Farfard Canadian growing mix No. 1-P, Agawam, Massachusetts, USA). Experimental plants were kept in a common garden under identical conditions until the start of the experiment. To impose the plant damage treatment we selected plants of similar height and stem density (40-45 cm tall, 2 stems/pot) and covered all of them (including plants receiving no herbivory) with a gauze bag. We then placed zero, 10 or 20 *Galerucella* adults inside each bag to produce the desired level of damage: no damage (no adults), medium damage (25-50% leaf area removed, 10 adults) and high damage (51-75% leaf area removed, 20 adults). After 5 days all beetles were collected and gauze bags were removed from all plants. Plants were immediately transported to each site and randomly positioned in each section of the experimental grid.

All insects for the study were collected in the Northern Montezuma Wetland Complex (Savannah, NY) on 16 June 2005, transported to the laboratory, individually counted and kept overnight on fresh purple loosestrife leaves. We released 600 beetles per site the next evening at the center of each experimental grid (x= 4m, y= 5m, medium damage treatment). Releases

were conducted at night to reduce beetle activity and dispersal.   Beetles were left at the release location in an open 250 ml container filled with fresh purple loosestrife stems. *Galerucella* show strong tendency to aggregate (Grevstad and Herzig 1997); therefore, we anticipated that beetles would disperse slowly, as purple loosestrife resources became depleted.

To assess the rate of colonization from the release point to each experimental plant, we counted the observed number of beetles per plant during 1-min intervals between 700 and 900 h daily from 18 June to 23 June 2005. Sites were visited randomly to avoid trends due to differences in beetle activity during the day. We did not differentiate between *G. calmariensis* and *G. pusilla* in the field as species are indistinguishable unless dissected.

Experimental Data Analyses: To evaluate if insects moved randomly, we compared the distribution of *Galerucella* for each plant attack treatment to the spatial distribution predicted by a simple diffusion model (Turchin 1998). Assuming random movement at a constant rate, insect distribution will be predicted by the diffusion equation:

$$\frac{\delta u(x,y,t)}{\delta t} = D\left(\frac{\delta^2 u}{\delta x^2} + \frac{\delta^2 u}{\delta y^2}\right) \tag{1}$$

where u(x,y,t) is the density distribution of marked insects at location (x,y), given that they have been moving for time t with diffusion rate D (Okubo 1980, Turchin 1998).   The solution to the diffusion equation is given by:

$$u(r,t) = \frac{No}{4\pi Dt}\exp\left[-\frac{r^2}{4Dt}\right] \tag{2}$$

where No is the number of organisms released and $r = \sqrt{x^2 + y^2}$ is the distance from the release point.

To test our hypothesis of simple diffusion, we estimated the mean diffusion coefficient $\overline{D}_i(t_i) = \overline{R}^2(t_i)/4t$ for each damage treatment at each sampling time t_i, where $\overline{R}^2(t_i)$ is the mean square displacement of released insects at time (t_i). We then estimated mean diffusion coefficient $\hat{D}$ for each damage treatment over all sampling dates and used it to generate the predicted density of resighted individuals at time t_i by plugging $\hat{D}$ into equation (2). Finally, we compared the predicted to the observed distribution of released *Galerucella* adults using a Kolmogorov-Smirnov test (Kareiva 1982, 1983, Turchin 1998).

The diffusion model assumes that there is no effect of directionality on insect movement; therefore, the expected mean displacement (ND) is zero. Alternatively, if insects were preferably moved towards damaged plants, we would expect the net displacement to differ from zero. To test this prediction we determined the net displacement of resighted *Galerucella* separately for the x- and y-axis and for each sampling date: $ND = \sum xC_{xt} / N_t$ (Cronin et al. 2000). Where x = distance along the x-axis, C_{xt} = number of insects resighted at distance x and time t and N_t = total number of insects resighted on time t. ND along the y-axis was calculated using a similar procedure. Net displacement departures from the origin at each sampling date were evaluated with separate *t* tests. The critical value was adjusted using the Bonferroni correction to achieve an overall error rate of 0.05.

Finally, to assess if the scale and shape of the dispersal kernel differed between plant damage treatments, we calculated mean dispersal distance and kurtosis for each section of the plot (no, medium and high damage).

Simulation model: We simulated *Galerucella* movement among purple loosestrife plants with varying host damage through a spatially explicit stochastic process. Simulated *Galerucella* insects were initially released at the center of a 500 x 500 m plot and at subsequent time steps insects chose whether to stay or leave their host plant, and if appropriate which plant to colonize next. Plants were selected according to an attraction probability generated by weighing the effects of distance, conspecific presence and plant damage. Plant attraction (A_d) decreased with distance (R) following and hyperbolic tangent function $A_d = \tanh(-aR^b)$ with parameters a = 0.000018 and b = 2 (Morales and Carlo 2006). Parameters were chosen such that the probability of colonizing a plant at 170 m is approximately 0.5, a relationship found by Grevstad and Herzig (1997). Plant attraction was higher when conspecifics were present (0.8 and 0.2 probability of colonizing plants with and without conspecifics, respectively) according to results from field releases of *Galerucella* which indicated that 86% of insect recaptures occurred on plants inhabited by conspecifics (Grevstad and Herzig 1997). Based on our experimental data, plants with low (<25%), medium (25 to 50%), high (51 to 75%) and extreme (>75%) damage level were assigned a probability of colonization of 0.15, 0.35, 0.50 and 0.15, respectively. Probabilities for each attraction factor were multiplied and standardized to sum up to one. This distribution was then sampled to determine which plant to visit next. After each time step the program recorded the position of each insect and updated the plant status. We assumed no plant regrowth, therefore plants that were not colonized kept their damage level, whereas plants that received > 100 adults were raised to the extreme damage level (0.15 probability of being chosen).

Simulation experiment: We followed a factorial design with two factors: insect density (10, 100 and 1000 released insects) and degree of purple loosestrife aggregation. Plant distribution was simulated using a Neyman-Scott process with 18 clusters and a total of 90 purple loosestrife plants per landscape. Location of parent plants was determined by a Poisson process by generating random x and y positions from a uniform distribution. A parent was chosen randomly and the position of the "daughter" plant followed a Weibull distribution with a scale parameter of two and the shape parameter determined according to the aggregation level desired. We used four shape parameters (0.01, 0.001, 0.0001, 0.00001) to generate landscapes with low to high levels of plant aggregation, respectively (Morales and Carlo 2006). We ran 15 replicates of each treatment combination for 30 simulated days.

The shape and scale of the dispersal kernel for each treatment was assessed via mean dispersal distance and kurtosis. We also assessed the dispersal kernel by fitting a Weibull distribution to dispersal distances using maximum likelihood. The shape of the Weibull distribution is flexible and therefore may approximate a variety of dispersal kernels. If the shape parameter equals two, then the kernel describes simple diffusion movement, a shape parameter of one describes an exponential fit, whereas a value lower than one indicates a fat tail (Morales and Carlo 2006). Finally, to evaluate if movement followed a simple diffusion process we estimated mean diffusion rate for each treatment and plug it in equation (2). The observed kernels from the simulation data were compared to predicted kernels from equation (2) with a Kolmogorov-Smirnov goodness of fit test (see Methods, experimental data analyses). All data analyses and simulations were run in S-plus 8.0 (Insightful Corporation 2007).

Results

Field Experiment: The mean number of observed *Galerucella* adults per plant was 4.86 ± 1.20. Mean number of observed adults did not vary among sites (F=0.61, P=0.54) or dates (F=0.33, P=0.9), but was significantly affected by damage treatment (F=27.47, P<0.001) with higher number of beetle observations on plants with medium damage (9.92 ± 0.07) than on plants with no (2.43 ± 0.008) or high damage (2.22 ± 0.008).

When the whole plot was analyzed together (without considering plant damage), *Galerucella* movement was adequately characterized by a simple diffusion process (Table 3.1). However, movement through each section of the plot (no, medium and high plant damage) did not follow a diffusion process (Table 3.1). Under the diffusion framework, the diffusion rate is assumed to be constant; yet in our case, it significantly decreased with time (F=88.87, P=1.68e-12; Fig. 3.1) and varied according to plant damage treatment (F=72.02, P=3.55e-15): mean diffusion rate was significantly lower in the medium damage section of the plot than in the no- and high-damage sections (Tukey's test, alpha=0.05, Table 3.1). Furthermore, the rate of decrease was dependent on damage treatment (interaction date x damage F=39.8, P=6.4e-11): diffusion rate decreased sharply in the no- and high-damage sections of the plot but was fairly constant in the medium damage section (Fig. 3.1).

Table 3.1. Mean diffusion rate (mean ± 2SE, n=3 sites) and fit of observed data to a simple diffusion model (Kolmogorov-Smirnov test) according to plant damage treatment. Significant differences of the means are indicated with different letters (Tukey's test, alpha=0.05).

Plant damage	Diffusion rate (m^2/day)	Kolmogorov-Smirnov Test	
		ks	P
Whole plot	0.58 ± 0.08	0.47	0.027
No damage	1.22 ± 0.28[a]	0.13	0.999
Medium damage	0.33 ± 0.08[b]	0.45	0.211
High damage	1.37 ± 0.36[a]	0.20	0.938

Average net displacement of *Galerucella* over the whole sampling period did not differ from the origin along the y-axis (F=0.24, P=0.93) but was marginally different from zero along the x-axis (F=3.05, P=0.052). During the first days of the experiment insects preferably moved towards the high damage section of the plot (to the right of the origin along the x-axis), but as un-damaged plants were colonized (by the fourth sampling dates all plants showed at least some *Galerucella* attack), insects moved equally in both directions from the point of origin (Fig. 3.2). Net displacement along the y-axis did not differ from zero on any of the sampling dates.
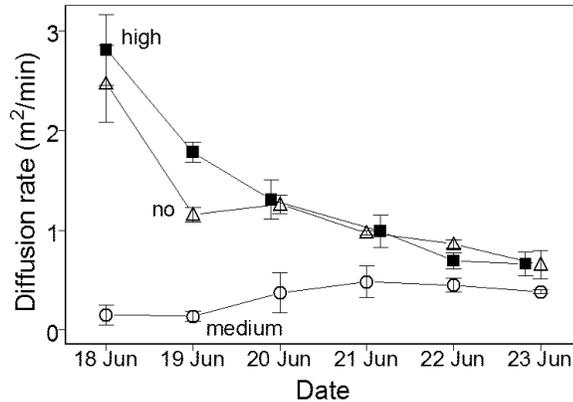
Figure 3.1. Mean diffusion rate according to sampling date and plant damage treatment: no damage (open triangles), medium damage= 25-50% leaf area removed (open circles), high damage=51-75% leaf area removed (filled squares). Data are mean ± 2SE, n=3 sites. Pairs of data points are staggered for clarity.

Mean dispersal distance increased with time (F=41.51, P=6e-14), and was higher in the no- and high-damage sections of the plot than in the medium damage section (plant damage: F=300, P<0.001, Tukey's test alpha=0.05). While mean dispersal distance in the no- and high- damage sections was relatively constant over all dates, mean dispersal in the medium damage section increased over the first four days before leveling out at a similar value of the no- and high-damage sections (plant damage x date interaction: F=10.74, P=3.8e-8, Fig. 3.3a). Damage explained 63.16% of variation in mean dispersal distance, while date explained 21.78% and the interaction explained 11.27%. Plant damage treatment explained 83.76% of the variation in kurtosis (F=15.47, P=0.004), with higher kurtosis in the high-damage section of the plot than in the no- and medium damage sections (Tukey's test alpha=0.05, Fig. 3.3b).
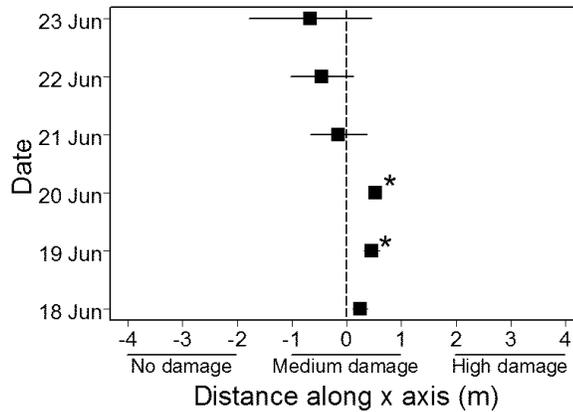
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Figure 3.2. Net displacement rate of *Galerucella* along the x-axis on each sampling date. Data points represent mean ± 2SE (n=3 sites). Asterisks denote dates when mean net displacement was significantly different from zero (separate t-tests adjusted with Bonferroni correction to achieve overall alpha=0.05).

Simulation Experiment: The shape and scale of dispersal kernels was significantly affected by *Galerucella* density and purple loosestrife aggregation. Mean dispersal distance was significantly affected by the level of purple loosestrife aggregation (F=5.43, P=0.0016), attaining its lower value in the least aggregated landscape (Tukey's test, alpha=0.05, Fig. 3.4a), but dispersal distance was not affected by *Galerucella* density (F=0.19, P=0.821) and the interaction between *Galerucella* density and host-plant aggregation (F=1.082, P=0.377). Variations in kurtosis were significantly affected by host-plant aggregation level (F=55.28, P<0.0001), insect density (F=107.91, P<0.0001) and the interaction between plant aggregation and insect density (F=21.47, P<0.0001). Kurtosis increased with number of insects released and was higher for the least aggregated landscape, although there was no

difference among the other plant aggregation levels (Tukey's test, alpha=0.05, Fig. 3.4b).
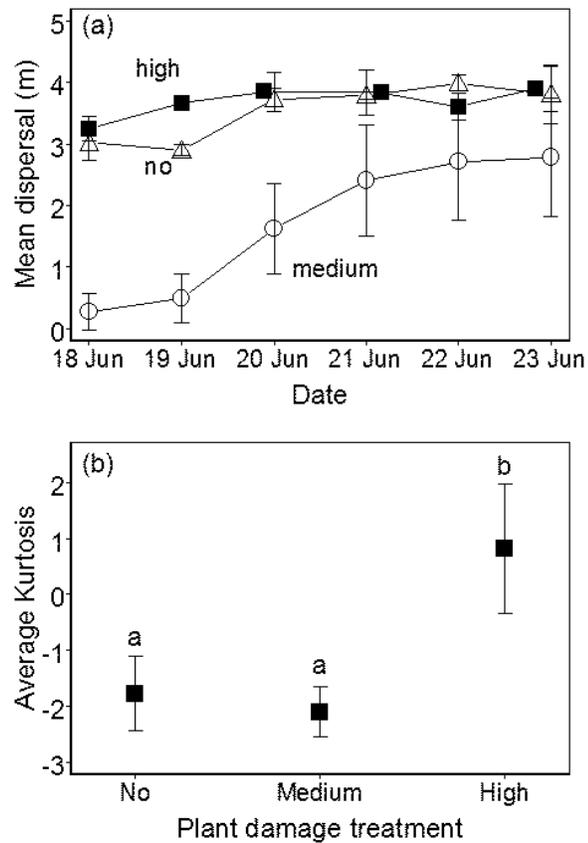
Figure 3.3. Mean dispersal distance in m (a) and kurtosis (b) according to plant damage treatment: no damage (open triangles), medium damage= 25-50% leaf area removed (open circles), high damage=51-75% leaf area removed (filled squares). Data represent mean ± 2SE, n=3 sites. Different letters represent significant differences of the means (Tukey's test, alpha=0.05). Pairs of data points are staggered for clarity.

Galerucella dispersal distances were well described by the Weibull distribution. Kolmogorov-Smirnov goodness of fit tests between the simulated

data and a theoretical distribution with the same parameters were not significant for all cases, indicating no differences between both distributions. The shape parameter of the fitted Weibull distributions ranged from 0.52 to 0.72, indicating dispersal kernels with fat tails. Shape parameter values tended to increase with increasing number of dispersers but showed no difference among levels of purple loosestrife aggregation.
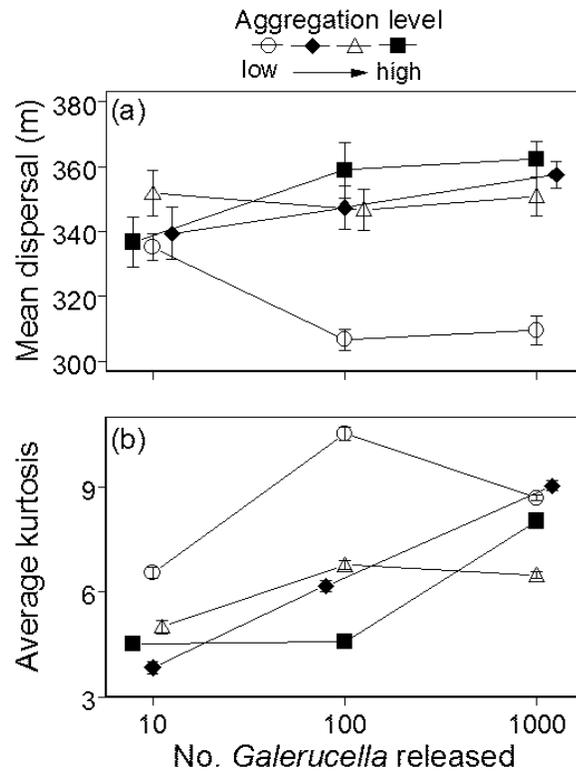

Figure 3.4. Mean dispersal distance in m (a) and kurtosis (b) of simulated *Galerucella* according to aggregation level and initial insect density. Data points represent mean ± 2SE (n=10 replicates). Data points are staggered for clarity.

Mean diffusion rate decreased with time but was not affected by purple loosestrife aggregation level or *Galerucella* density treatment (Fig. 3.5). Dispersal kernels calculated by plugging the average diffusion rate into equation (2) significantly differed from dispersal kernels calculated from the simulated data (P<0.0001 for all goodness of fit tests).
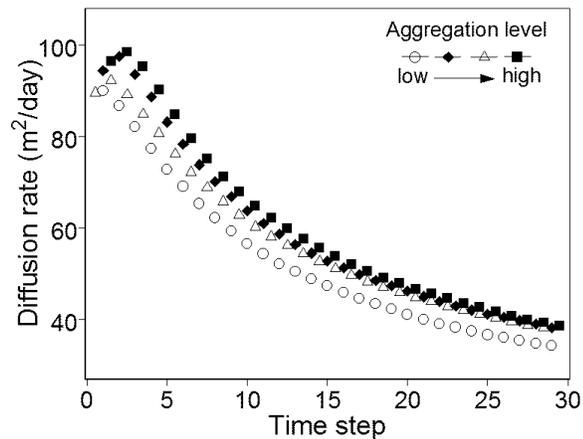
Figure 3.5. Change of mean diffusion rate over time in landscapes with different levels of aggregation. Results are shown for simulations with 1000 individuals. Results for other densities tested (10 and 100 individuals) showed a similar pattern. Data points are staggered for clarity.

Discussion

Our field and simulation results indicate that *Galerucella* movement cannot be approximated by a simple diffusion process. Although *Galerucella* movement over the whole experimental plot was well described by the diffusion model, its movement behavior differed in areas in response to variation in purple loosestrife damage levels. *Galerucella* preferred damaged over un-damaged plants (Fig. 3.2) and diffusion rates were higher in the non-

and high-damaged areas compared to the medium damage area (Fig. 3.1, Table 3.1). However, random colonization of non-damaged plants over the course of the study increased arrival of insects to newly colonized plants, resulting in a minor effect of plant damage by the end of the experiment.

The advantages of selecting previously attacked plants may be similar to those produced by conspecific aggregation, including overcoming plant defenses (Fordyce 2003, Franceschi et al. 2005), predator avoidance (Hunter 2000, Rohlfs and Hoffmeister 2004), increasing mating probabilities (Herzig and Root 1996) and it may aid insects in locating suitable host plants (Gilbert and Singer 1973). On the other hand, selection of previously attacked plants may negatively affect offspring (if adults oviposit) which would face increased intraspecific competition for limited plant resources. *Galerucella*'s aggregative behavior coupled with selection of attacked plants may further increase the propensity for local outbreaks leading to increased control of purple loosestrife. Extreme plant damage, on the other hand, may prompt dispersal allowing local rebound of purple loosestrife plants, but increasing the rate of spread of the biocontrol agent. Thus, the interplay between localized aggregated attack and resource-dependent dispersal may have significant consequences for biocontrol success.

The shape and scale of dispersal kernel can have important ecological consequences, influencing interactions with hosts and predators, coexistence with competitors and landscape persistence (Darrouzet-Nardi et al. 2006, Morales and Carlo 2006). *Galerucella* mean dispersal distance was lower in areas with medium plant damage compared to areas with no- and high damage, while diffusion rate followed the opposite pattern. Kurtosis is an indication of the shape of the dispersal kernel. Leptokurtic kernels (kurtosis

>0), characterized by a sharp peak and fatter tails, are common among different taxa and may arise through different mechanisms including differential movement rates among individuals, settling rate and boundary effects (Kareiva 1983, Cronin et al. 2000, Morales 2002). Our results indicate leptokurtic dispersal over the high damage areas but platykurtic (kurtosis < 0) dispersal in no- and medium damage areas. Leptokurtic dispersal over high plant damage areas is likely reflecting two types of behavior: organisms that settle on defoliated plants and those that engage in longer flights in search of suitable resources. Leptokurtosis can greatly increase the rate of spread due to its fatter and longer tails (Kot et al. 1996). Consequently, we expect lower spread rate in the non- and medium plant damage areas than in high plant damage areas, where kurtosis was higher. These differences may increase biocontrol success as insects with lower spread rate are more likely to build up high populations locally; whereas individuals in high damage areas with higher spread rate may colonize new patches of purple loosestrife thereby increasing the range over which purple loosestrife is potentially controlled.

Degree of aggregation of purple loosestrife plants is likely to vary according to the level of plant control: at early stages purple loosestrife is usually abundant and relatively unfragmented, but as biocontrol control progresses purple loosestrife density and patch size decrease (Blossey et al. 2001b), increasing plant clustering. Our simulation results indicate that differences in purple loosestrife distribution and insect density will promote different *Galerucella* dispersal behavior. As plants become more aggregated *Galerucella*'s mean dispersal distance increased, whereas kurtosis decreased. We expected mean dispersal distance to be lower in highly clustered landscapes because *Galerucella* has to cross less distance between purple

loosestrife plants. On the other hand, if plants within a cluster get severely defoliated *Galerucella* will have to cross over a longer distance to arrive at new less-defoliated patches. The dispersal kernels were leptoturkic (kurtosis > 0) for all aggregation and density levels tested, but kurtosis tended to increase with insect density and to decrease as purple loosestrife plants were more aggregated. Higher insect density causes higher local depletion of resources prompting more long and medium distance flights, which will likely result in dispersal kernels with fatter tails, and hence higher kurtosis, as the ones we observed. Moreover, lower mean dispersal in the least aggregated landscapes is likely to promote insect aggregation, which will lead to increased need to engage in long-distance flights resulting in dispersal kernels with fatter tails.

In both field and simulation experiments mean diffusion rate decreased with time, violating one of the assumptions of simple diffusion which states that diffusion rate is constant over time (Turchin 1998). Changes in diffusion rates have been reported for other organisms and may be caused by a series of factors, including weather variability (for example wind and temperature), density dependence and habitat heterogeneity (Kareiva 1983, Turchin 1998). Aggregation behavior and selection of damaged plants, which causes insects to settle down, are likely responsible for the observed decrease in diffusion rate. A decrease in diffusion rate may be of importance for biocontrol as insects rate of spread over time will be less than expected. However, our study, did not consider eventual long-distance dispersal, which *Galerucella* is capable of performing (Grevstad and Herzig 1997). However, high mortality associated with long flights (Grevstad and Herzig 1997) and within patch

movement behavior, may indicate that longer flights are rare and less successful than previously thought.

The simple diffusion model makes several simplifying assumptions, which do not reflect *Galerucella* behavior. For example, *Galerucella* aggregative behavior violates the assumption that insects are moving independently of each other (Turchin and Thoeny 1993). Our field experiment was short term and conducted at small spatial scales, but *Galerucella* are active from late April-July and have strong dispersal capabilities (Grevstad and Herzig 1997). Finally, we did not differentiate between both species, yet evidence indicates that they may have different dispersal abilities (Grevstad 2006, Dávalos and Blossey unpublished manuscript). A simulation model that incorporated *Galerucella*'s attraction to defoliated plants, and conspecifics, and the effect of distance, may increase the accuracy of our predictions.

Several simplifying assumptions were made to construct the simulation model. We assumed no purple loosestrife regrowth, but early in the season purple loosestrife can grow up to one cm per day, which affects resource availability for the insects. Also, we considered all matrix habitats equally unsuitable, yet *Galerucella* dispersal ability is influenced by matrix type (Dávalos and Blossey, unpublished manuscript). However, we consider that purple loosestrife regrowth and low-permeability matrices will only decrease the rate of spread, as *Galerucella* will encounter more food within a patch and will face higher resistance to cross an unsuitable habitat.

Our field and simulation experiments indicate that *Galerucella* is capable of adjusting its dispersal strategy depending on landscape characteristics and demographic conditions. Since dispersal can have a strong influence on population dynamics and on the distributional range of a

species, it is clear that the effects of plant aggregation and conspecific density may be of importance for purple loosestrife control. For instance, lower plant aggregation reduced dispersal distances likely increasing insect aggregation and hence increase local damage to purple loosestrife plants. On the other hand, higher insect density and higher host-plant damage produced dispersal kernels with fatter tails which will likely increase the range over which purple loosestrife is controlled. Thus the interaction between local and regional outbreaks is likely to increase purple loosestrife damage at the landscape level improving its control.

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CHAPTER 4

GUIDELINES FOR SUCCESSFUL CONTROL OF PURPLE LOOSESTRIFE

USING BIOLOGICAL CONTROL

Purple Loosestrife

Purple loosestrife (*Lythrum salicaria*) is a wetland herb introduced to North America from Europe and Asia in the early 1800s. Since its introduction purple loosestrife spread quickly throughout the continent and it is now present in the lower 48 US states and 6 Canadian provinces. Plants can form thick stands where other vegetation cannot grow. Invasion by purple loosestrife displaces native plants and can have negative impacts on amphibians and several bird species.

Control through traditional methods, such as chemical and mechanical techniques has been difficult because plants are long lived and can produce up to 2.7 million seeds that persist in the soil for several years. Additionally, purple loosestrife plants can regrow and spread from roots and stems. Failure to control purple loosestrife through traditional methods led to the development of a biological control program.

Biological Control Program

The biological control program started in the early 1990s with the introduction of four specialist beetles (Order: Coleoptera) into North America from Europe. The insects are a flower-feeding weevil (Family: Curculionidae), a root-feeding weevil (Family: Curculionidae) and two leaf-feeding beetles (Family: Chrysomelidae). These insects have been widely released in North

America, specially the leaf-feeding beetles which are easily reared and can be collected and redistributed from site to site.

The biological control program has been very successful at controlling purple loosestrife. At the majority of sites, the insects can completely defoliate patches of purple loosestrife, reduce plant height and reproductive success (for example, decrease the number of flower or seeds) and eventually kill the plant. After several years of attack, purple loosestrife density decreases allowing re-colonization of native plant species.

Leaf-feeding Beetles (*Galerucella calmariensis* and *Galerucella pusilla*, Family Chrysomelidae)

Natural History

Both species of leaf-feeding beetles share similar appearance and behavior. They are highly specific to purple loosestrife and they will not complete their development on any other host plant in North America. Leaf-feeding beetles are active from April to July (occasionally into September). Adults spend the winter in the leaf litter or soil surrounding purple loosestrife plants. In the spring, adults emerge and feed for a short period before mating. Females lay their eggs in batches on purple loosestrife leaves and stems. Newborn larvae (1st instar) crawl to the shoot tips to feed in buds where they are concealed from predators. Older larvae will feed on leaves and stems. After completing three instars larvae crawl down the stem and pupate in the soil or leaf litter surrounding the plant. In flooded areas, larvae pupate inside a spongy tissue (aerenchyma) created by the plant in response to inundation.

Development from egg to adult takes between 30 to 40 d and under favorable circumstances, a second generation may occur.

Of the four insect species introduced to control purple loosestrife, the leaf-feeding beetles are the most abundant and widespread and they cause the most visible damage. At successful sites the insects can defoliate entire purple loosestrife populations, reduce plant height, biomass and reproduction and eventually kill otherwise healthy plants. Usually, leaf-feeding beetles take 3 to 5 years to build up populations to levels where they can exert considerable damage to purple loosestrife plants.

Suggestions for releasing and redistributing leaf-beetles

Leaf-feeding beetles can establish and survive in many habitats as long as purple loosestrife is present, but several actions can be taken to increase their likelihood of establishing and increasing population size:

- Leaf-beetles will arrive inside a small container filled with purple loosestrife leaves. To release the insects simply remove all leaf material and insects and place it into a purple loosestrife patch, or shake it gently onto a purple loosestrife plant.

- All individuals should be released at a single location, unless a very large quantity of insects is released. Leaf-beetles like to aggregate, which increases their mating possibilities and reduces predation. Insects will disperse on their own as food (purple loosestrife plants) becomes scarce.

- Leaf-beetles should be released as soon as possible preferably during the early morning or late afternoon. Heat and stress due to transportation may cause insects to fly away reducing the number of

insects established at a site. If insects need to be stored, place containers into a refrigerator. Limit storage to 24 hours or less.

- There are no recommendations as to how many adults to release per unit area (acre). Release of biocontrol agents is seen as an inoculation. Beetle populations will grow over time and impact on plants in the year of the release may be limited.

- There is no minimum size for biocontrol to be effective.   Biocontrol will work fine as long as there are at least 50 plants.   Insects should be released in open uplands or wetlands, and not in small forest openings.

- Insects collected in the spring will feed and reproduce at the release site producing visible effects in the same year. Insects collected in the summer will not lay eggs and will feed only for a short period before migrating to overwintering sites. Their effects will only be seen during the following spring. We recommend spring releases with a minimum of 1,000 adults. For summer releases at least 5,000 adults are recommended.

- Leaf-beetle eggs and larvae can be predated by several species, including wasps, ground beetles, myrid bugs and ladybugs. The effect of predation can be most severe at initial releases, when the leaf-beetle populations are still small. If predation prevents establishment, try multiple releases, increase the number of insects released at a given time or protect insects at time of release with a cage.

- Leaf-beetles will establish in both flooded and well-drained sites, but will do better in flooded areas. Flooded areas provide a refuge from predators and it is likely that purple loosestrife quality (as a food source for the beetles) is better. The best sites are those that provide a

combination of flooded and dry areas. Dry areas provide over-wintering sites, while flooded areas sustain larger populations of leaf-feeding beetles. Winter and spring flooding is common at many purple loosestrife sites. Leaf-feeding beetles have adapted to these conditions. Some winterkill will occur but summer floods are more devastating to beetle populations.

- Avoid releasing insects in shady spots or close to border lines, especially adjacent forests.

- Leaf-beetles may leave purple loosestrife patches that have been extremely defoliated in search for new food. As purple loosestrife re-grows the insects will re-colonize the area. To facilitate migration between sites, maintain connections (ex. water bodies, marshes) between purple loosestrife sites. It is unlikely for leaf-feeding beetles to cross even small areas of closed forests and the likelihood of establishing in purple loosestrife patches surrounded by forests is lower than in open sites.

- Purple loosestrife control increases when leaf-beetles are used in combination with root-feeding weevils.

Monitoring The Effect

It is very important to check regularly how the beetles are doing: did they establish, have they dispersed and what is their effect on purple loosestrife. This information will increase our knowledge of the interaction of insects and plants and will help in developing appropriate management recommendations. It is important to record the information systematically: using the same methods and gathering the same data on each occasion and

site. This way information gathered at different localities around the country can be easily compared and compiled. A monitoring protocol can be found at www.invasiveplants.net