

INDIRECT EFFECTS OF HERBIVORY ON PLANT–POLLINATOR INTERACTIONS IN INVASIVE *LYTHRUM SALICARIA*¹

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- *Premise of the study:* Herbivory can affect a plant's fitness in a variety of ways, including modifying the biotic interactions of the plant. In particular, when herbivory influences floral display, we hypothesize that pollinator visitation will be altered accordingly. Here we studied the indirect effects of feeding by two beetles, *Neogalerucella calmariensis* and *N. pusilla*, released as a biological control, on plant–pollinator interactions and fitness in the invasive plant, purple loosestrife (*Lythrum salicaria*).
- *Methods:* Two herbivory treatments (ambient and simulated) were applied to plants in a naturally occurring population of purple loosestrife. During flowering, traits of plants in the treatment and control groups were recorded. Data on pollinator visitation behavior was then collected after intense larval herbivory had ended.
- *Key results:* Plants exposed to herbivory treatments produced more flowers and inflorescences but flowered significantly later than those in the control group. Moreover, we found a significant, positive association of herbivory with the number of flowers probed by bumblebees and with the number of times a foraging pollinator moved among inflorescences on a single plant. No differences in female fitness (fruit or seed production) were detected.
- *Conclusions:* We conclude that herbivore-mediated differences in floral display traits impacted pollinator visitation behavior. However, as we discuss, differences in pollinator visitation did not translate into detectable differences in female reproductive success. We postulate that herbivory could influence other unmeasured aspects of fitness, such as seed quality or the number of seeds sired.

Key words: herbivory; invasive plant; Lythraceae; *Lythrum salicaria*; multispecies interactions; *Neogalerucella calmariensis*; *Neogalerucella pusilla*; pollination.

Interactions with pollinators have been established as a defining feature of the evolutionary history of flowering plants (Vamosi and Vamosi, 2010). Yet, interactions among plants and their pollinators are, by necessity, embedded in the context of a wider ecological community (Miller and Travis, 1996; Barber et al., 2012). A spate of reviews urges those in the field to consider the net effects of species on one another, rather than focusing on plants and pollinators in isolation (Wootton, 1994; Strauss and Irwin, 2004). Indeed, the strength and response to selection predicted by pairwise relationships among species are likely to deviate when multispecies interactions are considered (Miller and Travis, 1996; Strauss and Irwin, 2004). For example, in a study of *Erysimum mediohispanicum* (Brassicaceae),

positive pollinator-mediated selection on floral traits (such as floral display height, flower number, and flower size) was counteracted by the tendency of ungulate herbivores to browse plants with larger floral displays (Gómez, 2003). Moreover, a meta-analysis suggested that the harmful impacts of enemies on plants can be reduced through interactions with mutualists (Morris et al., 2007).

Studies that have examined plant–pollinator interactions in the context of interactions with other community members such as herbivores, seed predators, dispersal agents, and mycorrhizae are rare (Strauss and Irwin, 2004). At the same time, it is increasingly clear that such interactions can influence pollinator-mediated interactions, either directly, through, for example, predation on pollinators (Dukas, 2005) or indirectly, through the modification of floral traits that influence pollinator visitation (e.g., McCall and Irwin, 2006). Recent research has revealed that even underground fungal associations can impact pollinator visitation and behavior (e.g., Strauss, 1997; Cahill et al., 2008).

Herbivory affects pollinator-mediated fitness by altering plant traits that function to attract pollinators (reviewed by Strauss and Irwin, 2004). In previous studies, herbivory has been shown to affect the number of flowers produced (Barber et al., 2012), flower size (Steets and Ashman, 2004), flowering phenology (Pellegrino and Musacchio, 2006), the size and quantity of pollen (Lehtilä and Strauss, 1999), and nectar volume (Halpern et al., 2010) and composition (Adler et al., 2006).

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Such effects may arise through a variety of mechanisms, such as a loss of resource availability caused by damage to leaf or root tissues (Mutikainen and Delph, 1996; Zamora et al., 1999; Poveda et al., 2003). Florivory can also negatively impact flower appearance (Karban and Strauss, 1993), leading to increased flower abortion rates (Krupnick and Weis, 1998). Further, pollinator visitation may be negatively affected when herbivory induces the production of volatile organic compounds, which may alter floral scent (Kessler et al., 2011), or leach into nectar (Raguso, 2004). In addition to its influence on individual plant traits, herbivory can alter the density of plant populations and, in doing so, may negatively affect plant–pollinator interactions and the amount of conspecific pollen transferred (Vázquez and Simberloff, 2004).

Following root and/or leaf herbivory, the number and/or duration of floral visits by pollinators have been found to decrease (Kessler et al., 2011), increase (Barber et al., 2011), or remain unchanged (Hladun and Adler, 2009), depending on how herbivory alters floral traits. For example, feeding by thrips on flowers of *Erigeron glaucus* (Asteraceae) alters the appearance of petals and reduces pollinator visitation (Karban and Strauss, 1993). Lehtilä and Strauss (1997) reported that altered flower production in *Raphanus raphanistrum* (Brassicaceae) as a result of leaf herbivory by *Pieris rapae* larvae likely explains decreased pollinator visitation to plants undergoing herbivory treatment.

In contrast, somewhat counter-intuitively, some species appear to become more attractive to pollinators following herbivory. For example, experimental manipulation of root herbivory has been associated with higher pollinator visitation (Poveda et al., 2003). Another surprising response to herbivory is an increase in the production of flowers and inflorescences that results in higher plant fitness, a phenomenon that has been termed “over-compensation” (Paige and Whitham, 1987). Overcompensation and compensation, once thought to be quite rare, have now been identified in a number of species (Venecz and Aarssen, 1998; Brody and Irwin, 2012).

When herbivory leads to an increase in the number of flowers and inflorescences produced, pollinator visitation may be affected. Large display size has been widely shown to be associated with higher pollinator visitation rates (reviewed by Ohashi and Yahara, 2001). On the other hand, large displays are also associated with more flowers probed per foraging bout, which can increase the rate of geitonogamy (i.e., between flower self-fertilization) (de Jong et al., 1993). Finally, changes to the pollinator visitation rate does not necessarily translate into changes to fruit and seed set, most likely because of resource limitation, which can itself be a direct effect of herbivory (Poveda et al., 2003).

In the present study, we investigated the potential for herbivory by the beetles *Neogalerucella californiensis* L. and *N. pusilla* Duft. (Coleoptera; Chrysomelidae), introduced in Ontario in 1992 to control the invasive plant species purple loosestrife (*Lythrum salicaria*), to alter plant traits that affect plant–pollinator interactions and reproductive success. Herbivory by *Neogalerucella* beetles on *L. salicaria* has been demonstrated to modify multiple plant traits, including height, inflorescence number, total inflorescence length, flowering phenology, and the number of flowers produced (Blossey and Schat, 1997; Katovich et al., 2001; Landis et al., 2003; Schat and Blossey, 2005). We hypothesized that, due to changes in phenology and floral display traits that have been previously shown to accompany herbivore damage in *L. salicaria*, pollinator visitation patterns, and consequently seed and fruit set, would be altered in plants experiencing experimental herbivory manipulation.

MATERIALS AND METHODS

Study species—*Lythrum salicaria* L. (Lythraceae) is a perennial, herbaceous plant. Multiple stems, which can reach 0.5–2.7 m tall, arise from common underground rootstock annually (Blossey, 1995b). This showy species depends on animal pollinators, and its primary mode of reproduction is sexual (Eckert and Barrett, 1992). Red-purple flowers have petals that are ~7–12 mm long (Gleason and Cronquist, 1991) and are arranged on spikes on the main and axillary stems (Mal et al., 1992). Flowers are perfect and individual plants exhibit one of three stylar morphs (short-, mid-, or long-morph) (Mal et al., 1992).

Biological control program—Due to concerns about the negative effects of *L. salicaria* on North American ecosystems, two species of beetle in the genus *Neogalerucella*, *N. californiensis* and *N. pusilla*, were released in the early 1990s in Canada and the United States for the purpose of biological control (Malecki et al., 1993). In eastern North America, adult *Neogalerucella* beetles emerge between late May to early June (Lindgren et al., 2002) after overwintering in the soil (Lindgren, 1999) or leaf litter (Blossey, 1995a; Lindgren et al., 2002). Emerging adults feed on leaves and meristems for approximately 1 wk before the onset of oviposition (Blossey, 1995a). Individual females lay up to 10–12 eggs per day over a period of approximately 8–10 wk, after which the adults die. Larvae feed on meristems in addition to leaves and various other tissues (Lindgren et al., 2002). Before returning to the soil or leaf litter for winter diapause, newly emerged adults feed on *L. salicaria* (Blossey, 1995a). There is normally a single generation per season; however, in some years and for some locations (including our eastern Ontario population), there may be a second generation (Kok et al., 1992).

Study site—All fieldwork was conducted at Cow Island Marsh (N 44°34'9.70", W 076°19'13.30") on Lake Opinicon on the lands of the Queen's University Biological Station in eastern Ontario, Canada. The study population covered an area of approximately 10000 m², with approximately 3.7 ± 0.79 (SE) plants per m² (averaged over 60 random 0.38 m² plots).

Herbivory—During the second week of June 2011, 105 plants of *L. salicaria* were chosen and marked for inclusion in the study. We note that while it is fairly straightforward to identify all the stems of a plant in the field, because of the growth pattern of purple loosestrife (new shoots arise as sprouts from a dense underwater root), it is possible that some stems of some plants, especially new shoots that arose after our covers were applied, may have been inadvertently left out of the cover treatment. All plants included in the study were approximately the same height, with no visible meristem damage. Study plants were selected to be at least 1 m apart to reduce the probability of including more than one stem from a single plant (Haldane, 1936). Plants were visually inspected for *Neogalerucella*, and all detected adults, larvae and egg masses were physically removed. Each plant was then covered with a herbivore-exclusion sleeve made of no-see-um netting (Rose e Dee Ltd., Toronto, Ontario, Canada), and randomly assigned to one of three groups ($N = 35$ per group): control (no herbivores added), ambient herbivory, or simulated herbivory.

Herbivory treatments began during the third week of June 2011. In the control group, covers were temporarily lifted at least once a week so that plants could be inspected for any beetles that had found a way to breach the cover. Any beetles detected were immediately removed. This continued until pollinator observations were conducted several weeks later. For plants in the ambient herbivory treatment, seven *Neogalerucella* larvae (chosen because it was the average number of larvae counted on a sample of plants in the study population), collected from populations in close proximity to our study site, were added to the plant's stems, leaves, and primary developing meristem before the replacement of the cover. All larvae were collected from the study population at Cow Island Marsh or from a second population at Indian Lake, located approximately 1.5 km away from the study population. Larvae were transported in Petri dishes with *L. salicaria* leaves and placed on treatment plants within a few hours. For the simulated herbivory treatment, once a week, for a period of 3 wk, the apical meristem of each plant was clipped with scissors and a leaf disk (approximately 6 mm in diameter) was taken from each leaf that was large enough to have a leaf disk removed. At the end of 3 wk, the average percentage leaf damage (visual estimate) of simulated herbivory plants (average % leaf damage = 33.8, $N = 35$) was similar to that of a set of naturally damaged (via herbivory) plants (average % leaf damage = 32.2, $N = 16$) that were intermingled with the study population (t test on log-transformed data: $F_{1,49} = 2.75$, $P = 0.104$). Due to the loss (by deer trampling) of two of the control plants early in the study, two replacement plants were haphazardly chosen 10 d after the last

sleeve had been applied to the rest of the study plants, cleaned of larvae, and covered with herbivore-exclusion sleeves.

Covers were removed from all plants in the study population on 8 and 9 July 2011, soon after the start of flowering, to perform the pollinator observations and allow plants to set seed. All covers were removed at the same time to avoid covering study plants for different lengths of time. A second generation of adult beetles was observed after the covers were removed. After the detection of this second generation, plants in the study population were inspected at least once a week, and all detectable eggs, larvae, and adults were removed.

Pollinator observations—Pollinator observations were conducted daily from 26 July through 5 August 2011 on sunny or mainly sunny days without strong wind between ~9:00–17:00 hours (following King and Sargent, 2012), for a total of 10 d. Each observation period consisted of a randomly assigned observer using a hand-held digital device to record the identity and foraging behavior of pollinators visiting a single plant over a 30-min period. Pollinators were classified by sight into five taxonomic groups (*Bombus* spp., solitary bees, *Apis mellifera* [honeybees], syrphid flies, and wasps) (following Da Silva et al., 2013). A visit was defined as a pollinator contacting the stigma and/or anthers of a flower (following King and Sargent, 2012). A foraging bout was defined as a pollinator visiting one or more flowers on the focal plant. A total of 289 pollinator observation periods (144.5 hours) were conducted. Before each observation period, the following information was recorded about the focal plant: the number of inflorescences, the number of open flowers per inflorescence, and the height of the tallest stem on the target plant (cm). The sequence of plants chosen for each day's pollinator observations were randomized and Pearson χ^2 tests indicate that there was no significant bias toward any treatment with respect to observer ($\chi^2 = 8.68$, $df = 12$, $p = 0.730$), date ($\chi^2 = 13.7$, $df = 20$, $p = 0.847$) or time of observation ($\chi^2 = 16.5$, $df = 14$, $p = 0.286$).

Due to the delayed onset of flowering in some plants and damage (browsing and/or trampling by deer, etc.) to other plants, the sample size was reduced from 105 to 83 plants (control: $N = 26$, ambient herbivory: $N = 29$, simulated herbivory: $N = 28$).

Fruit and seed set—Fruit were collected in the fall of 2011 and stored in coin envelopes in a laboratory at the University of Ottawa. The height (cm) of the tallest fruiting stem on each plant was measured prior to fruit removal and the total length of all fruiting stems was measured with a digital caliper (Mitutoyo, Aurora, Illinois, USA). The number of fruits per plant was recorded. Following Colautti et al., (2010), the number of seeds per fruit was averaged among five randomly chosen fruits per plant, as exhaustive seed counting was impractical. Seeds were counted using a custom written automated computer program, which counts seeds from a digital image, implemented in the program MATLAB (Mathworks, 2009). The number of seeds per plant was estimated by multiplying the number of fruits per plant by the average number of seeds per fruit. The sample size for fruit and seed production was 80 plants (control: $N = 24$, ambient: $N = 27$, simulated: $N = 29$) due to loss of additional study plants in the weeks between pollinator observations and fruits collection (due to e.g., browsing and/or trampling by deer).

Statistical analyses—Individual plants were used as the unit of replication for all analyses. Pollinator visitation and fruit and seed production were analyzed as plant averages using one-way ANOVA, weighted by the number of observations per individual plant, with treatment as a fixed effect. Where necessary, data were transformed to meet the assumptions of parametric statistics (Quinn and Keough, 2002). In some instances, nonparametric tests (e.g., Kruskal–Wallis) were used to compare groups when data failed to meet the assumption of normality. However, since ANOVA is known to be robust to violations of normality (Quinn and Keough, 2002) and Kruskal–Wallis and ANOVA provided quantitatively similar results, only ANOVA results are reported. Post hoc testing was conducted using Tukey's honestly significant difference (HSD) test when significant differences were detected among treatments.

Least-squares regression (or, when residuals were not normally distributed, Spearman's rank correlation) was used to examine the relationships among continuous plant traits (i.e., number of flowers, number of inflorescences, and height) and various metrics of pollinator visitation. Regression and correlation analyses were used because ANCOVA (with plant trait values as covariates) was not deemed to be an appropriate test, as the covariates were confounded by treatment. All analyses were performed in the program JMP 10.0 (SAS Institute, 2012).

RESULTS

Direct effects of herbivory—Prior to the experimental manipulation, there were no significant differences in average plant height (cm) among treatments (Fig. 1, $F_{2,103} = 1.57$, $P = 0.212$). However, there were significant differences in mean height (cm) during the pollinator observations. Specifically, plants in the ambient herbivory treatment were significantly shorter than either the control or simulated herbivory plants (Fig. 1, $F_{2,78} = 6.19$, $P = 0.0032$). The difference between the ambient and the control, while less prominent, was still detectable when fruits were harvested at the end of the growing season (Fig. 1, $F_{2,79} = 3.68$, $P = 0.0297$). Mean plant height did not significantly differ between plants in the control group and the simulated herbivory group at any point.

The number of inflorescences on a plant, as measured at the start of each pollinator observation period, differed significantly among treatments (Fig. 2, $F_{2,80} = 11.6$, $P < 0.0001$), with plants in the control group having significantly fewer inflorescences than in either herbivory treatment. Treatment also had a significant effect on the number of infructescences produced per plant ($F_{2,77} = 5.66$, $P = 0.0051$).

There was a significant effect of treatment on the date of first flower (Fig. 3A, $F_{2,73} = 6.19$, $P = 0.003$) and the mean number of flowers open during pollinator observations (Fig. 3B, $F_{2,80} = 5.14$, $P = 0.0079$). The onset of flowering was significantly earlier in the control group, by an average of 6 d, than for either the ambient or simulated herbivory treatments. During pollinator observations, plants in the ambient and simulated herbivory treatments tended to have more open flowers than plants in the control group, but only the difference between the simulated treatment and the control group was significant.

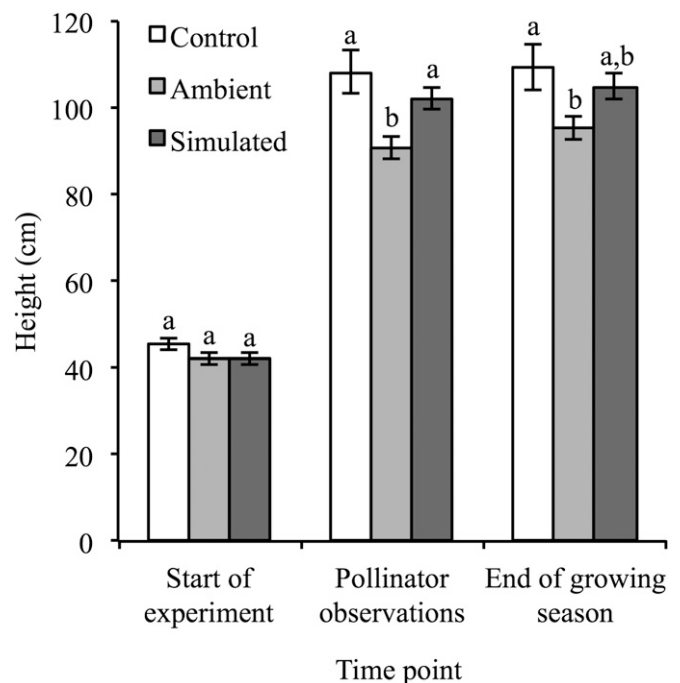


Fig. 1. Mean (± 1 SE) height of *Lythrum salicaria* plants among a control group and two herbivory treatments at three points in time. Note: lettering is specific to each time point and does not make comparisons among time points. Bars that are not connected by the same letter are significantly different ($P < 0.05$).

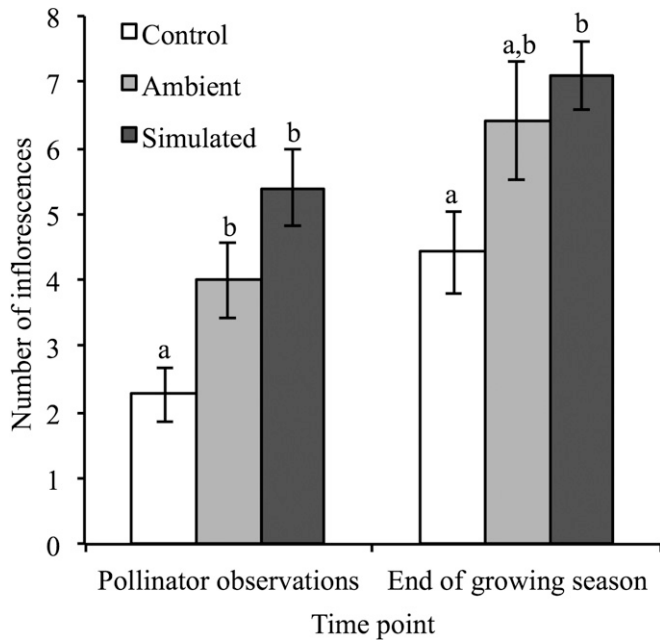


Fig. 2. Mean (± 1 SE) number of inflorescences on *Lythrum salicaria* plants in a control group and two herbivory treatments during pollinator observations and by the end of the growing season. The total number of inflorescences produced by the end of the growing season was inferred from the total number of fruiting stalks collected. Note: lettering is specific to each time point and does not make comparisons among time points. Bars that are not connected by the same letter are significantly different ($P < 0.05$).

Pollinator community—*Bombus* spp. comprised 89.0%, 85.4%, and 90.0% of the total number of pollinators that visited control, ambient herbivory, and simulated herbivory treatment plants, respectively. Other species of pollinators visiting plants in the study population included solitary bees (1.5–4.8% of all visitors), syrphid flies (3.4–6.4% of all visitors), and wasps (2.8–3.1% of all visitors). Honeybees made up a small percentage of the visiting pollinator community (0.4% and 0.6% of visitors to the control group and simulated herbivory treatment, respectively), did not visit the ambient herbivory treatment plants, and were only recorded on four of 11 observation days. We were unable to identify the remaining 0.4–0.6% of pollinators.

Pollinator visitation—When all pollinator species were considered, there were no significant differences among treatment groups in terms of the number of foraging bouts (defined as a pollinator visiting ≥ 1 flowers on a focal plant) per 30 min ($F_{2,80} = 2.17$, $P = 0.121$). There was a marginally significant trend toward plants in the ambient and simulated herbivory treatments having more flowers probed per observation period than plants in the control group (Fig. 4, $F_{2,80} = 2.92$, $P = 0.0597$). In addition, there was a marginally significant difference among treatments in the number of flowers probed by a pollinator while visiting a particular plant ($F_{2,71} = 3.02$, $P = 0.0554$), which is likely to be correlated with the degree of geitonogamy. On average, the number of flowers probed per foraging bout was lowest in the control group and highest in the mechanical herbivory treatment. Treatment also had a significant effect on the mean number of switches among inflorescences per foraging bout. Specifically, pollinators made significantly more switches (per foraging bout) among inflorescences in the ambient and simulated herbivory treatments, relative to the control ($F_{2,71} = 9.58$, $P = 0.0002$).

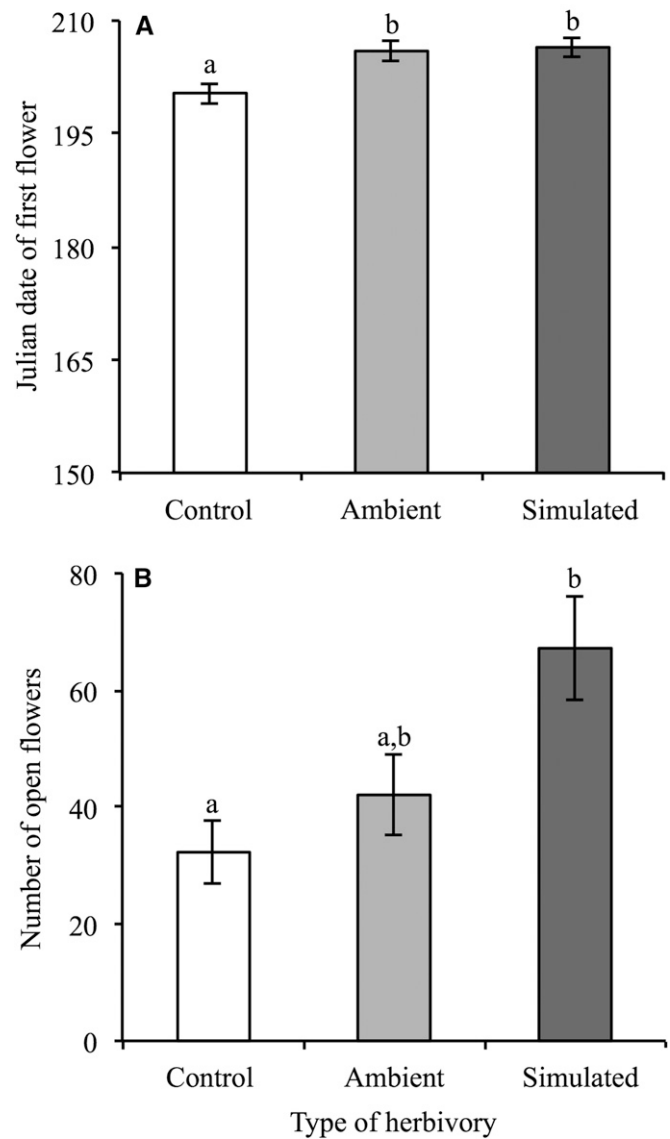


Fig. 3. Mean (± 1 SE) (A) Julian date of first flower ($N = 76$) and (B) number of open flowers during pollinator observations ($N = 83$) in *Lythrum salicaria* plants in a control group and two herbivory treatments. Note: lettering is specific to each panel. Bars that are not connected by the same letter are significantly different ($P < 0.05$).

When only visits by *Bombus* were considered (the vast majority of visiting species), there was a significant effect of treatment on the total number of flowers probed per observation period, with control plants receiving significantly fewer probed flowers than plants in the simulated herbivory treatment (Fig. 4, $F_{2,80} = 3.45$, $P = 0.0366$). In addition, treatment had a significant effect on the mean number of switches among inflorescences per foraging bout. During foraging bouts, *Bombus* pollinators made significantly more switches among inflorescences in the ambient and simulated herbivory treatments, relative to the control ($F_{2,70} = 7.82$, $P = 0.0009$). Treatment did not influence any other aspects of *Bombus* visitation behavior (i.e., number of foraging bouts per 30 min, number flowers probed by a pollinator when visiting a particular plant; all $P > 0.05$).

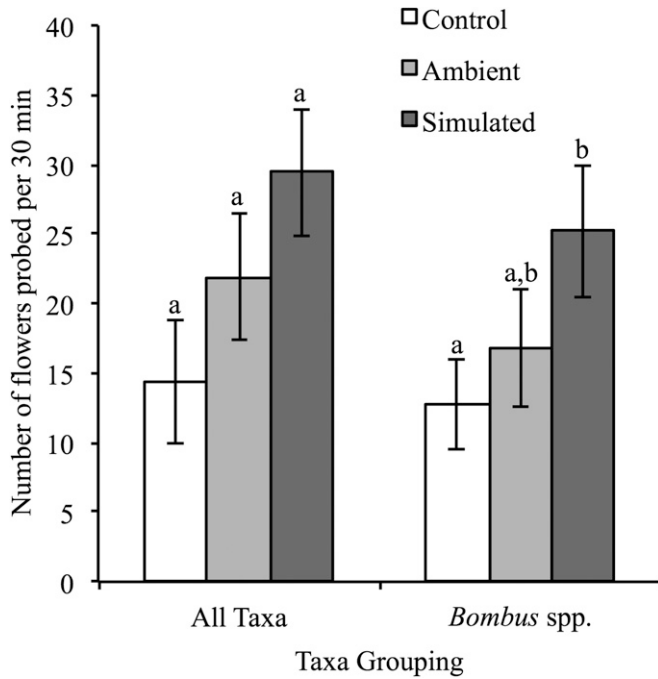


Fig. 4. Weighted mean (± 1 SE) number of flowers probed, grouped by all pollinator taxa (first set of columns) and by *Bombus* spp. only (second set of columns), per observation period, for the control group and two herbivory treatments. Note: lettering is specific to each taxa grouping and does not make comparisons among the taxa groupings. Bars that are not connected by the same letter are significantly different ($P < 0.05$).

Indirect effects of herbivory on pollinator visitation—There were significant relationships among the three measured plant characteristics (plant height, the number of flowers per plant, and the number of inflorescences per plant) and various aspects of pollinator visitation behavior (Table 1). The average number of open flowers per plant explained the greatest amount of variation in the number of foraging bouts and flowers probed per observation period. However, interpreting relationships between these plant traits and pollinator visitation is difficult due

TABLE 1. Test statistics for least-squares regression and Spearman’s rank correlations among plant traits and aspects of pollinator visitation.

Aspect of pollinator visitation	No. flowers	No. inflorescences	Height
No. of foraging bouts per 30 min ^a	$R^2 = 0.579$ $F_{1,81} = 112$ $P < 0.0001$	$R^2 = 0.470$ $F_{1,81} = 71.8$ $P < 0.0001$	$R^2 = 0.314$ $F_{1,79} = 36.1$ $P < 0.0001$
No. of flowers probed per 30 min ^a	$R^2 = 0.739$ $F_{1,81} = 229$ $P < 0.0001$	$R^2 = 0.601$ $F_{1,81} = 122$ $P < 0.0001$	$R^2 = 0.308$ $F_{1,79} = 35.1$ $P < 0.0001$
No. of flowers probed per foraging bout ^b	$R^2 = 0.411$ $F_{1,72} = 50.1$ $P < 0.0001$	$R^2 = 0.295$ $F_{1,72} = 30.2$ $P < 0.0001$	$R^2 = 0.078$ $F_{1,71} = 6.00$ $P = 0.0167$
No. of switches among inflorescences per foraging bout ^a	$R^2 = 0.501$ $F_{(1,72)} = 72.3$ $P < 0.0001$	$\rho = 0.842$ $P < 0.0001$	$\rho = 0.333$ $P = 0.0040$

^aResponse variable was square-root transformed for least-squares regressions.

^bResponse variable was log-transformed for least-squares regressions.

to multicollinearity among the plant traits. There were significant positive linear relationships between the number of flowers (square root transformed to achieve normality of residuals) and plant height ($R^2 = 0.264$, $F_{1,79} = 28.4$, $P < 0.0001$), the number of flowers and the number of inflorescences ($R^2 = 0.778$, $F_{1,81} = 284$, $P < 0.0001$), and the number of inflorescences (square-root transformed to achieve normality of residuals) and plant height ($R^2 = 0.145$, $F_{1,79} = 13.4$, $P = 0.0005$).

Fruit and seed production—No statistically significant differences were detected among treatments in the mean number of fruits per plant ($F_{2,77} = 1.87$, $P = 0.161$), the mean number of seeds per five randomly chosen fruits on a plant ($F_{2,77} = 0.289$, $P = 0.750$), or the (extrapolated) mean number of seeds per plant ($F_{2,77} = 1.56$, $P = 0.216$). There were significant positive relationships between plant height and the number of fruits per plant ($R^2 = 0.400$, $F_{2,78} = 52.1$, $P < 0.0001$), the number of seeds per fruit ($R^2 = 0.107$, $F_{2,78} = 9.31$, $P = 0.0031$), and the number of seeds per plant ($R^2 = 0.471$, $F_{2,78} = 69.5$, $P < 0.0001$).

DISCUSSION

If we want to better understand how natural selection shapes a plant’s response to herbivory, we must consider how specific responses impact all aspects of a plant’s environment, including interactions with mutualists. We predicted that a response that elicits a change in floral display, as has been identified in a number of flowering plant species, would lead to changes in plant–pollinator interactions that would result in fitness effects. Our results support some, but not all, of these predictions. Specifically, we found that herbivory, both simulated and ambient, directly affected several aspects of the floral display of *L. salicaria*. Plants in the herbivory treatment groups tended to flower later and produced more flowers and more inflorescences than plants in the control group. These phenotypic differences were associated with differences in the behavior of pollinators visiting plants from the three experimental groups. Specifically, plants in the herbivory treatments tended to have more flowers probed per pollinator visit than those in the control group, especially when the analysis focused on the main group of pollinators (bumblebees). Furthermore, pollinators visiting plants in the treatment groups tended to move among inflorescences more often than those visiting plants in the control group. None of these findings translated into detectable differences in female fitness.

Previous studies have reported an increase in pollinator visitation to plants damaged by herbivores. For example, browsing by ungulates has been demonstrated to enhance maternal and paternal fitness in *Ipomopsis aggregata* (Gronemeyer et al., 1997). Furthermore, in two separate studies, plants that had experienced root herbivory received more pollinator visits than those that had not (Poveda et al., 2003; Barber et al., 2011). In the present study, differences in floral display among groups, specifically flower number, explained the greatest amount of variation in pollinator visitation. This result is consistent with previous work demonstrating a positive relationship between the number of open flowers on a plant and the number of flowers visited during a pollinator visit (reviewed by Ohashi and Yahara, 2001).

Despite significant differences in pollinator visitation among treatments and a greater number of inflorescences in treatment plants, we did not detect a significant difference in fruit or seed production among treatments. Poveda et al. (2003) also

reported significantly increased pollinator visitation, as an indirect effect of herbivory, without subsequent significant differences in seed set. Indeed, it is not unusual for studies reporting differences in visitation rate to fail to find concomitant changes in seed or fruit set (Totland, 2004). There are several possible explanations for this finding. First, if baseline levels of pollinator visitation were such that all plants received enough pollen to fertilize nearly all ovules (i.e., pollen limitation was minimal, or absent), then higher rates of visitation in the herbivory treatment groups would not result in an overall difference in seed set. Second, although herbivory can deplete resources, which can restrict ovule production, plants in the herbivory treatments may have allotted a greater proportion of resources to ovule production to maintain fitness achieved via female function (Lehtilä and Strauss, 1999). Third, undetected differences in fruit abortion rates could have offset any increased fruit production in the herbivory treatments, as studies have found that fruit abortion rates are significantly higher in branches subjected to defoliation, relative to nondefoliated branches, in some species (Stephenson, 1980; Niesenbaum, 1996). Our results are also consistent with a relatively small, nonmanipulative study that reported no effect of *Neogalerucella* herbivory on the number of *L. salicaria* seeds per fruit (Katovich et al., 2001).

Differences among the treatments in the number of flowers probed, both overall and per visit, could have important effects on plant fitness by altering gene flow (via pollen transfer and receipt) (Harder et al., 2001). The probability of transferring self-pollen to stigmas rises with the number of flowers visited on a plant during a single foraging bout (de Jong et al., 1993). Thus, female reproductive success may have been negatively impacted by increased floral display size, as *L. salicaria* is partially self-compatible (Stout, 1923). Moreover, *L. salicaria* suffers from inbreeding depression; seeds produced via self-pollination are likely to be of reduced quality (O'Neil, 1992). Indeed, in a study examining the impact of artificial clipping on seed set in *L. salicaria*, Venecz and Aarssen (1998) found that germination rates were significantly lower for seeds from clipped than unclipped plants, suggesting that the clipped plants produced lower quality seeds. Although they were not able to identify a mechanism, if, as our study suggests, geitonogamy is more likely in clipped plants, this could provide an explanation for the reduction in seed quality.

Male fitness, which was not explicitly measured in our study, may be influenced by pollinator visitation patterns, through pollen export and pollen discounting (Ohashi and Yahara, 2001). Rush et al. (1995), in a study of rates of pollen removal in wild radish, reported a significant positive linear relationship between the number of pollinator visits received and the amount of pollen exported from individual flowers. Hence, increased pollinator visitation may have influenced male fitness in the herbivory treatments by increasing total pollen export and the number of seeds sired per plant (Harder et al., 2001). Moreover, the effect of pollinator visitation on pollen export may differ among the three style morphs of *L. salicaria*. For example, in another tristylous species (*Pontederia cordata*), additional visits by pollinators to individual flowers only influenced the amount of pollen removed in midlevel anthers (Harder and Barrett, 1993). Pollen limitation in *L. salicaria* has been shown previously to vary with style morph—long-morphed plants typically exhibit greater pollen limitation than either short- or mid-styled plants (Thomsen, 2014).

Our results demonstrate that *Neogalerucella* beetle herbivory directly affects the vegetative and reproductive traits of *L. salicaria*,

which is consistent with findings of previous studies (Blossey and Schat, 1997; Katovich et al., 2001; Landis et al., 2003; Schat and Blossey, 2005). The simulated and ambient herbivory treatments had similar effects on traits, indicating that plant responses to *Neogalerucella* herbivory are likely induced by tissue and meristem damage, rather than something specific to beetle herbivory (Blossey and Schat, 1997; Van Zandt and Agrawal, 2004; Voelckel and Baldwin, 2004; Schat and Blossey, 2005). Our study also demonstrates that herbivory by *Neogalerucella* has the potential to indirectly affect pollinator-mediated fitness in *L. salicaria*, as demonstrated by the numerous differences in pollinator visitation patterns to plants subjected to the herbivory treatments compared with the control plants. The effect of increased pollinator visitation was likely mediated by the increase in inflorescence and flower production following herbivory. We did not detect an effect of herbivory on fruit or seed production. However, additional, yet unmeasured effects of increased pollinator visitation on *L. salicaria* may be present, such as reduced male (i.e., through increased pollen discounting) or female (i.e., reduced seed quality through inbreeding depression) reproductive success. Our study underscores the importance of considering and investigating multispecies interactions when considering the overall impacts of herbivory on plants.

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