

A Study of the Impact of an Introduced Herbivore on Pollinator-Mediated
Interactions and Female Fitness in *Lythrum salicaria*

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Abstract

Herbivory can have many effects on plant fitness, including altering plant-pollinator interactions and sexual reproduction in angiosperms. Pollinator-mediated interactions may be impacted when herbivores alter plant traits, such as floral display size, that can influence pollinator visitation rates, and, ultimately, the reproductive component of plant fitness. Here I describe an investigation into the indirect effects of feeding by beetles released as a biological control agent, *Galerucella californiensis* and *G. pusilla*, on plant-pollinator interactions and reproductive output in the invasive plant *Lythrum salicaria* L. (purple loosestrife). During the summer of 2011, three treatments (low, ambient and mechanical herbivory) were applied to 105 plants during the pre-flowering period of growth. At the onset of flowering, a series of pollinator observations were conducted over the course of approximately 1.5 weeks. Several aspects of floral display were affected by the herbivory treatments, including increased inflorescence and flower production in the ambient and mechanical herbivory treatments, relative to the low herbivory treatment. Treatment type did not have a significant effect on the number of pollinator foraging bouts, but had marginally significant effects on the number of flowers probed per pollinator foraging bout and per 30-minutes. Moreover, treatment had a significant effect on the number of switches among the inflorescences on a single plant. I discuss the possibility that the differences in pollinator visitation were mediated by differences in the architecture and the size of floral display. There were no detectable differences in fruit or seed production (i.e., female fitness) among treatments. However, as I discuss, differences in pollinator visitation may affect other unmeasured aspects of fitness, such as the level of inbreeding or the number of seeds sired through male function.

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CHAPTER 1: General Introduction and Overview

General Introduction and Overview

Multispecies interactions

All organisms coexist and interact with other species in their natural environments. Yet, ecological studies frequently focus on pairwise interactions and generally ignore the importance of multispecies interactions. As a result, we have a limited ability to make predictions about the net effects of one species on another (Wootton 1994, Miller and Travis 1996, Strauss and Irwin 2004, Barber et al. 2012). As noted by Strauss and Irwin (2004), the strength and response to selection predicted by pairwise relationships among species, which are most often studied, might deviate when multispecies interactions are considered (also see Miller and Travis 1996). For example, in *Erysimum mediohispanicum* (Brassicaceae), pollinator-mediated selection on floral traits (such as floral display height, flower number, and flower size) is counter-balanced by the tendency of ungulate herbivores to browse on plants with a larger floral display (Gómez 2003). Indeed, the effects of one organism on a focal species may be heightened or decreased by altered interactions between the focal species and its enemies or mutualists within the community (Morris et al. 2007, Barber et al. 2012). A recent meta-analysis suggests that the harmful impacts of enemies on plants can be lessened by interactions between plants and mutualist taxa (Morris et al. 2007). In the case of plant-pollinator interactions, herbivory can alter plant traits that influence pollinator behaviour and, in doing so, have effects on sexual reproduction and fitness (Strauss 1997, Strauss and Irwin 2004).

Effects of herbivory on plant reproduction

Herbivory can have direct effects on multiple components of plant fitness, either by reducing the probability of survival (Rausher and Feeny 1980, Strauss 1991, Brody et al. 2007) or by altering some other aspect of plant performance, such as rates of photosynthesis (Oleksyn et al. 1998, Zangerl et al. 2002), growth (Karban and Strauss 1993, Nötzold et al. 1998, Schat and Blossey 2005), and root:shoot biomass allocation (Vranjic and Gullan 1990, Karban and Strauss 1993), which can impact plant fitness (reviewed by Strauss and Irwin 2004). Herbivores can also affect male and female fitness, either by inducing a reduction in allocation towards male and/or female traits, and therefore the quality or quantity of gametes (i.e., pollen and ovules) (Strauss 1997, Lehtilä and Strauss 1999); by consuming male and/or female gametes (Krupnick and Weis 1999); or by consuming progeny (i.e., seeds or fruits) (Strauss and Irwin 2004).

In addition to its direct impacts on fitness, herbivory can alter plant traits that influence pollinator behaviour, which may indirectly affect pollinator-mediated fitness (Strauss 1997, Strauss and Irwin 2004). For example, herbivory may reduce pollinator visitation rates and/or the duration of visits to individual flowers if floral traits are negatively affected, such as through a reduction in flower size (Karban and Strauss 1993, Strauss et al. 1996, Lehtilä and Strauss 1997, Strauss 1997, Lehtilä and Strauss 1999, Mothershead and Marquis 2000, Steets and Ashman 2004, Barber et al. 2011), reduced numbers of flowers produced (Lehtilä and Strauss 1997, Adler et al. 2001, Hambäck 2001, Suárez et al. 2009, Barber et al. 2011, Barber et al. 2012), degraded flower appearance (Karban and Strauss 1993), modified floral scent (Kessler et al. 2011), or altered nectar composition and/or volume (Krupnick et al. 1999, Adler et al. 2006, Halpern et al. 2010). In contrast, pollinator visitation could increase where plants respond to herbivory with a change in floral traits that

positively influences pollinator attraction such as through increased nectar production (Poveda et al. 2003, Barber et al. 2011) or overcompensation in flower production (Schlichting and Levin 1984, Gronemeyer et al. 1997). An altered rate of pollinator visitation is expected to impact male and female fitness differently, due to the differential importance of pollen export and import to these two aspects of fitness (Strauss 1997, Krupnick and Weis 1999). Specifically, male fitness should be more sensitive to rates of pollen export, whereas resource acquisition more commonly limits female fitness (Bateman 1948, Willson and Burley 1983, Stanton et al. 1986).

Effects of herbivory on female and male fitness

Herbivory can directly limit female fitness by reducing ovary and ovule production (via altered resource availability) (Hambäck 2001, Sharaf and Price 2004), or by damaging those ovaries that are produced (Krupnick and Weis 1998). Herbivory can limit female reproduction indirectly if rates of pollinator visitation, and therefore pollen receipt, decline (Strauss 1997). For example, a reduction in the number of pollinator visitors may result in the pollen limitation of fruit and seed set (i.e., fruit and seed set is limited by access to mates), or increase the degree of pre-existing pollen limitation (Krupnick and Weis 1999, Mothershead and Marquis 2000, Kessler et al. 2011), which is not uncommon in plants (Bierzychudek 1981, Burd 1994, Ashman et al. 2004, Knight et al. 2005). Another potential indirect effect of herbivory may occur if herbivory leads to an increase in the number of open flowers (e.g., Schlichting and Levin 1984, Gronemeyer et al. 1997), which can result in more flowers visited during a given foraging bout (reviewed by Ohashi and Yahara 2001). The more flowers that are visited during a foraging bout the higher the degree of geitonogamy (selfing due to “the transfer of pollen between flowers on the same plant” (de Jong et al. 1993, p. 321)) (de Jong et al. 1993, reviewed by Harder et al. 2001, Ohashi and Yahara 2001)

and the lower the rate of outcrossing (Barrett et al. 1994, Harder and Barrett 1995). Thus, as noted by Krupnick and Weis (1999), altered rates of geitonogamy can have important consequences for female function by reducing offspring quality if inbreeding depression is present within a population. The degree of geitonogamy depends on the presence and strength of prezygotic barriers. For example, outcrossed pollen develops pollen tubes faster than, and has a siring advantage over, self-derived pollen in *Decodon verticillatus* (L.) Elliott. (Lythraceae) (Eckert and Allen 1997).

As with female function, herbivory can also impact male fitness directly and indirectly. Herbivory can lead to a reduction in pollen production (Frazee and Marquis 1994, Quesada et al. 1995, Strauss et al. 1996, Lehtilä and Strauss 1999), size (Quesada et al. 1995, Lehtilä and Strauss 1999), and competitive ability (Quesada et al. 1995, Mutikainen and Delph 1996). Altered pollinator behaviour, in response to herbivory, can impact male function via effects on rates of pollen export and discounting. For example, a reduction in the number of pollinators visiting a flower will decrease the amount of pollen exported (Klinkhamer et al. 1989, Krupnick and Weis 1999). However, male fitness can also be negatively impacted via different avenues of pollen loss if each pollinator visits more flowers per plant (reviewed by Biernaskie and Cartar 2004), as occurs in plants with more open flowers (reviewed by Ohashi and Yahara 2001). As the number of flowers visited per plant increases, so does the probability of pollen discounting, defined as pollen being unavailable for outcrossing due to deposition on self-stigmas (Harder and Wilson 1998, Harder et al. 2001). Finally, pollen may be groomed by pollinators, and therefore unavailable for outcrossing, in between visits to flowers on a plant (Thomson 1986, Harder 1990).

Due to the discussed potential impacts of pollinator visitation patterns, both male and female fitness should be maximized when a plant attracts many pollinators that each visit

few flowers, because this will limit inbreeding and increase pollen export (i.e., reduce geitonogamy and pollen discounting) (Klinkhamer et al. 1994, Harder et al. 2001, Biernaskie and Cartar 2004). However, male fitness is more limited by pollen export (i.e., access to mates), whereas the limitations to female fitness are more likely due to access to resources, according to Bateman's Principle (Bateman 1948, Willson and Burley 1983, Stanton et al. 1986). Thus, altered pollinator visitation, as an indirect effect of herbivory, may affect male fitness more than female fitness (Strauss et al. 1996, Krupnick and Weis 1999). For instance, following one visit to a flower by a pollinator, the proportion of ovules fertilized is much greater than the proportion of pollen exported for outcrossing (Bell 1985). Furthermore, when pollen grains are deposited on receptive stigmas they must compete with other males for access to ovules (Mutikainen and Delph 1996, Lehtilä and Strauss 1999). The barrier faced by males of access to mates (due to insufficient pollinator visitation) may be exacerbated for males exposed to herbivory, as feeding damage can decrease rates of pollen tube growth and thus reduce competitive ability in comparison to pollen from undamaged plants (Mutikainen and Delph 1996).

One caveat to the above is that females can also be limited by access to mates (Burd 1994), as demonstrated by the high frequency of pollen limitation of fruit and seed set in the wild (Bierzuchudek 1981, Burd 1994, Ashman et al. 2004, Knight et al. 2005). This effect may be exacerbated when plants suffer from damage imposed by herbivores. For example, Kessler et al. (2011) demonstrated that the seed set of *Solanum peruvianum* L. was limited by reduced pollinator visitation as an indirect effect of herbivory. Moreover, it has been shown that floral herbivory can reduce the number of pollen grains deposited on stigmas in *Isomeris arborea* (Capparaceae), although this did not result in pollen limitation of fruit set (Krupnick and Weis 1999). In addition, modified pollinator visitation rates, resulting from

herbivory, can affect both sexes, or it can negatively affect one sex while having no impact on the other (Krupnick and Weis 1999). In spite of the fact that herbivory can have dramatically different impacts on male and female fitness, it is only relatively recently that the indirect effects of herbivory on male and female fitness, via plant-pollinator interactions, have been considered in concert (Strauss 1997, Strauss et al. 2001). Gronemeyer et al. (1997) reported that herbivory by ungulates on *Ipomopsis aggregata* (Polemoniaceae) (scarlet gilia) resulted in increased flower production and an increase in male and female fitness; individuals damaged by ungulate feeding sired approximately twice the number of progeny (i.e., increased male fitness) and produced significantly more fruit (i.e., increased female fitness) than plants not subjected to ungulate herbivory. However, due to reduced pollinator visitation to *Isomeris arborea* in response to herbivory, pollen export from individual flowers was decreased by approximately 50% compared to pollen export from flowers on undamaged plants, whereas fruit set was not significantly affected by reduced pollinator visitation (Krupnick and Weis 1999).

Research Questions and Study System

In the United States, invasive species pose threats to many native taxa and annually cost the economy billions of dollars (Pimentel et al. 2005). Biological control agents have been successfully used to mitigate the impacts of many invasive species (reviewed by DeBach and Rosen 1991). However, a failure to consider the ecological and evolutionary ramifications and the mechanisms through which biological control agents suppress their targets may result in unexpected and harmful consequences or limit the effectiveness of the biological control in the introduced range (Corey and Myers 2000, Simberloff 2012). For example, releases of the Indian mongoose (*Herpestes auropunctatus*), intended to control

rats on islands (including, but not limited to, Hawaii, Jamaica, and Puerto Rico) has resulted in many detrimental effects, including the spread of disease (reviewed by Pimentel et al. 2005) and, due to a lack of host specificity towards rats, predation on native animals (reviewed by Pimentel et al. 2005, Simberloff 2012).

Since herbivory can affect plant-pollinator interactions, and thus the reproductive component of fitness in plants, such multispecies interactions may be a mechanism through which herbivorous biological control agents impact the fitness of target invasive plant species (Swope and Parker 2010, Swope and Parker 2012). For example, while herbivorous biological control agents can reduce the competitive ability of invasive plants (e.g., reduce height, reduce biomass, etc. (Blossey and Schat 1997, Schat and Blossey 2005)), they may affect plant reproduction by altering pollinator-mediated interactions. In my thesis I investigate the potential for a biological control agent (two congener *Galerucella* beetle species) to indirectly affect plant-pollinator interactions and thereby female fitness (fruit and seed production) in the invasive plant *Lythrum salicaria* L. (Lythraceae) (purple loosestrife).

L. salicaria has migrated and established across North America since its initial introduction to the US northeast from Eurasia in the 1800s (Stuckey 1980, Thompson et al. 1987, Blossey et al. 2001). The impacts of *L. salicaria* on native ecosystems remains unclear; one body of research indicates that *L. salicaria* can outcompete native wetland plants (Gaudet and Keddy 1988, Mal et al. 1997, Weihe and Neely 1997), thereby reducing species diversity in wetlands (Thompson et al. 1987, Farnsworth and Ellis 2001, Hovick et al. 2011), while other research demonstrates that *L. salicaria* is not more competitive than native plants (Denoth and Myers 2007) and does not reduce species diversity (Treberg and Husband 1999, Farnsworth and Ellis 2001, Hager and Vinebrooke 2004, Houlahan and Findlay 2004, and reviews by Anderson 1995, Hager and McCoy 1998, Lavoie 2010).

Several methods have been used in an effort to control *L. salicaria* populations including fire, hand-pulling, herbicides, alteration of water levels, and mowing (reviewed by Thompson et al. 1987, Malecki et al. 1993, Blossey et al. 2001). However, these methods may damage ecosystems (e.g., herbicides) and are not cost-efficient or effective at large scales (reviewed by Thompson et al. 1987, Malecki et al. 1993, Blossey et al. 2001).

One hypothesis to explain the success of *L. salicaria* in North America is that it has escaped its natural enemies (enemy release hypothesis, also known as the escape-from-enemy hypothesis (Crawley 1986, Lawton and Brown 1986, Keane and Crawley 2002, Wolfe 2002)), thereby giving it a competitive advantage in the introduced range (Thompson et al. 1987, Malecki et al. 1993, Hunt-Joshi et al. 2004). Furthermore, the evolution of increased competitive ability (EICA) hypothesis proposes that, if a tradeoff exists between defensive and competitive traits, selection in the introduced range will favor increased allocation of resources to growth and/or reproduction (Blossey and Nötzold 1995). In 1992 the Canadian and U.S. governments approved the release of several taxa of non-native herbivorous beetles, which are natural enemies of, and share the same native range as, *L. salicaria*, to use as biological control agents against the invasive plant (Malecki et al. 1993). The taxa released in the Canadian province of Ontario include two congener beetles, *Galerucella californiensis* L. and *G. pusilla* Duftschmid (Coleoptera: Chrysomelidae), and a root mining weevil *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae). However, in Ontario *Galerucella* spp. populations were successfully established, whereas releases of *H. transversovittatus* failed to establish any viable populations (Lindgren et al. 2002).

Galerucella herbivory has been shown to reduce plant height (Blossey and Schat 1997, Schat and Blossey 2005), decrease total biomass (Blossey and Schat 1997), increase

the degree of branching and number of inflorescences (Schat and Blossey 2005), delay flowering (Schat and Blossey 2005), reduce the overall flowering period (Schat and Blossey 2005), reduce the number of flowers produced (Landis et al. 2003), lower inflorescence biomass (Schat and Blossey 2005), decrease inflorescence length (Katovich et al. 2001, Schat and Blossey 2005), and decrease seed production per plant (Katovich et al. 2001). While a number of studies have investigated the effects of *Galerucella* herbivory on *L. salicaria*, a literature search revealed no studies examining whether feeding by *Galerucella* affects pollinator-mediated interactions in *L. salicaria*.

The main aim of this thesis was to investigate multispecies interactions and their effects on pollinator-mediated fitness in an invasive species (*L. salicaria*) following the introduction of a non-native herbivore (the beetle, *Galerucella*). Specifically I ask:

1. Given that herbivory has been shown to affect floral display in other species, what impacts does *Galerucella* feeding have on the floral display of *L. salicaria*?
2. Abundant studies have established that an altered floral display can affect rates of pollinator visitation. If *Galerucella* feeding alters the floral display, what are the effects on pollinator visitation to *L. salicaria*?
3. If plant-pollinator interactions are altered by damage caused by *Galerucella* herbivory, then what are the effects on fruit and seed production in *L. salicaria*?

As noted above, herbivores can alter interactions between plants and their mutualists. Therefore, the strength of selection on plants due to herbivory may differ from that predicted by considering only interactions between plants and herbivores (Strauss and Irwin 2004). The work described in my thesis adds to the relatively limited body of literature on the indirect effects of herbivores on interactions between plants and pollinators (Strauss 1997, reviewed by Strauss and Irwin 2004).

References

- Adler, L. S., R. Karban, and S. Y. Strauss. 2001. Direct and indirect effects of alkaloids on plant fitness via herbivory and pollination. *Ecology* **82**:2032-2044.
- Adler, L. S., M. Wink, M. Distl, and A. J. Lentz. 2006. Leaf herbivory and nutrients increase nectar alkaloids. *Ecology Letters* **9**:960-967.
- Anderson, M. G. 1995. Interactions between *Lythrum salicaria* and native organisms: A critical review. *Environmental Management* **19**:225-231.
- Ashman, T. L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, S. J. Mazer, R. J. Mitchell, M. T. Morgan, and W. G. Wilson. 2004. Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology* **85**:2408-2421.
- Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* **2**:349-368.
- Barber, N. A., L. S. Adler, and H. L. Bernardo. 2011. Effects of above- and belowground herbivory on growth, pollination, and reproduction in cucumber. *Oecologia* **165**:377-386.
- Barber, N. A., L. S. Adler, N. Theis, R. V. Hazzard, and E. T. Kiers. 2012. Herbivory reduces plant interactions with above- and belowground antagonists and mutualists. *Ecology* **93**:1560-1570.
- Barrett, S. C. H., L. D. Harder, and W. W. Cole. 1994. Effects of flower number and position on self-fertilization in experimental populations of *Eichhornia paniculata* (Pontederiaceae). *Functional Ecology* **8**:526-535.
- Bell, G. 1985. On the function of flowers. *Proceedings of the Royal Society of London B: Biological Sciences* **224**:223-265.
- Biernaskie, J. M. and R. V. Cartar. 2004. Variation in rate of nectar production depends on floral display size: A pollinator manipulation hypothesis. *Functional Ecology* **18**:125-129.
- Bierzychudek, P. 1981. Pollinator limitation of plant reproductive effort. *The American Naturalist* **117**:838-840.
- Blossey, B., L. C. Skinner, and J. Taylor. 2001. Impact and management of purple loosestrife (*Lythrum salicaria*) in North America. *Biodiversity and Conservation* **10**:1787-1807.
- Blossey, B. and R. Nötzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: A hypothesis. *Journal of Ecology* **83**:887-889.

- Blossey, B. and M. Schat. 1997. Performance of *Galerucella californiensis* (Coleoptera: Chrysomelidae) on different North American populations of purple loosestrife. *Environmental Entomology* **26**:439-445.
- Brody, A. K., M. V. Price, and N. M. Waser. 2007. Life-history consequences of vegetative damage in scarlet gilia, a monocarpic plant. *Oikos* **116**:975-985.
- Burd, M. 1994. Bateman principle and plant reproduction: The role of pollen limitation in fruit and seed set. *Botanical Review* **60**:83-139.
- Cory, J. S. and J. H. Myers. 2000. Direct and indirect ecological effects of biological control. *Trends in Ecology & Evolution* **15**:137-139.
- Crawley, M. J. 1986. The population biology of invaders. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **314**:711-731.
- de Jong, T. J., N. M. Waser, and P. G. L. Klinkhamer. 1993. Geitonogamy: The neglected side of selfing. *Trends in Ecology & Evolution* **8**:321-325.
- Delph, L. F., M. H. Johannsson, and A. G. Stephenson. 1997. How environmental factors affect pollen performance: Ecological and evolutionary perspectives. *Ecology* **78**:1632-1639.
- Denoth, M. and J. H. Myers. 2007. Competition between *Lythrum salicaria* and a rare species: Combining evidence from experiments and long-term monitoring. *Plant Ecology* **191**:153-161.
- Eckert, C. G. and M. Allen. 1997. Cryptic self-incompatibility in tristylous *Decodon verticillatus* (Lythraceae). *American Journal of Botany* **84**:1391-1397.
- Farnsworth, E. and D. Ellis. 2001. Is purple loosestrife (*Lythrum salicaria*) an invasive threat to freshwater wetlands? Conflicting evidence from several ecological metrics. *Wetlands* **21**:199-209.
- Frazee, J. E. and R. J. Marquis. 1994. Environmental contribution to floral trait variation in *Chamaecrista fasciculata* (Fabaceae, Caesalpinoideae). *American Journal of Botany* **81**:206-215.
- Gaudet, C. L. and P. A. Keddy. 1988. A comparative approach to predicting competitive ability from plant traits. *Nature* **334**:242-243.
- Gómez, J. M. 2003. Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erisimum mediohispanicum*: Consequences for plant specialization. *The American Naturalist* **162**:242-256.
- Gronemeyer, P. A., B. J. Dilger, J. L. Bouzat, and K. N. Paige. 1997. The effects of herbivory on paternal fitness in scarlet gilia: Better moms also make better pops. *The American Naturalist* **150**:592-602.

- Hager, H. A. and K. D. McCoy. 1998. The implications of accepting untested hypotheses: A review of the effects of purple loosestrife (*Lythrum salicaria*) in North America. *Biodiversity and Conservation* **7**:1069-1079.
- Hager, H. A. and R. D. Vinebrooke. 2004. Positive relationships between invasive purple loosestrife (*Lythrum salicaria*) and plant species diversity and abundance in Minnesota wetlands. *Canadian Journal of Botany* **82**:763-773.
- Halpern, S. L., L. S. Adler, and M. Wink. 2010. Leaf herbivory and drought stress affect floral attractive and defensive traits in *Nicotiana quadrivalvis*. *Oecologia* **163**:961-971.
- Hambäck, P. A. 2001. Direct and indirect effects of herbivory: Feeding by spittlebugs affects pollinator visitation rates and seedset of *Rudbeckia hirta*. *Ecoscience* **8**:45-50.
- Harder, L. D. 1990. Behavioral responses by bumble bees to variation in pollen availability. *Oecologia* **85**:41-47.
- Harder, L. D. and S. C. H. Barrett. 1995. Mating cost of large floral displays in hermaphrodite plants. *Nature* **373**:512-515.
- Harder, L. D. and W. G. Wilson. 1998. A clarification of pollen discounting and its joint effects with inbreeding depression on mating system evolution. *The American Naturalist* **152**:684-695.
- Harder, L. D., N. M. Williams, C. Y. Jordan, and W. A. Nelson. 2001. The effects of floral design and display on pollinator economics and pollen dispersal. Pages 297-317 in L. Chittka and J. D. Thomson, editors. *Cognitive ecology of pollination: Animal behaviour and floral evolution*. Cambridge University Press, West Nyack, NY, USA.
- Houlahan, J. E. and C. S. Findlay. 2004. Effect of invasive plant species on temperate wetland plant diversity. *Conservation Biology* **18**:1132-1138.
- Hovick, S. M., D. E. Bunker, C. J. Peterson, and W. P. Carson. 2011. Purple loosestrife suppresses plant species colonization far more than broad-leaved cattail: Experimental evidence with plant community implications. *Journal of Ecology* **99**:225-234.
- Hunt-Joshi, T. R., B. Blossey, and R. B. Root. 2004. Root and leaf herbivory on *Lythrum salicaria*: Implications for plant performance and communities. *Ecological Applications* **14**:1574-1589.
- Karban, R. and S. Y. Strauss. 1993. Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. *Ecology* **74**:39-46.
- Katovich, E. J. S., D. W. Ragsdale, L. C. Skinner, and R. L. Becker. 2001. Effect of *Galerucella* spp. feeding on seed production in purple loosestrife. *Weed Science* **49**:190-194.

- Keane, R. M. and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* **17**:164-170.
- Kessler, A., R. Halitschke, and K. Poveda. 2011. Herbivory-mediated pollinator limitation: Negative impacts of induced volatiles on plant-pollinator interactions. *Ecology* **92**:1769-1780.
- Klinkhamer, P. G. L. and T. J. de Jong. 1990. Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). *Oikos* **57**:399-405.
- Klinkhamer, P. G. L., T. J. de Jong, and G. J. de Bruyn. 1989. Plant size and pollinator visitation in *Cynoglossum officinale*. *Oikos* **54**:201-204.
- Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T. L. Ashman. 2005. Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology, Evolution, and Systematics* **36**:467-497.
- Krupnick, G. A. and A. E. Weis. 1998. Floral herbivore effect on the sex expression of an andromonoecious plant, *Isomeris arborea* (Capparaceae). *Plant Ecology* **134**:151-162.
- Krupnick, G. A. and A. E. Weis. 1999. The effect of floral herbivory on male and female reproductive success in *Isomeris arborea*. *Ecology* **80**:135-149.
- Krupnick, G. A., A. E. Weis, and D. R. Campbell. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology* **80**:125-134.
- Landis, D. A., D. C. Sebolt, M. J. Haas, and M. Klepinger. 2003. Establishment and impact of *Galerucella californiensis* L. (Coleoptera: Chrysomelidae) on *Lythrum salicaria* L. and associated plant communities in Michigan. *Biological Control* **28**:78-91.
- Lavoie, C. 2010. Should we care about purple loosestrife? The history of an invasive plant in North America. *Biological Invasions* **12**:1967-1999.
- Lawton, J. H. and K. C. Brown. 1986. The population and community ecology of invading insects. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **314**:607-617.
- Lehtilä, K. and S. Y. Strauss. 1997. Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. *Oecologia* **111**:396-403.
- Lehtilä, K. and S. Y. Strauss. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. *Ecology* **80**:116-124.

- Lindgren, C. J., J. Corrigan, and R. A. De Clerck-Floate. 2002. *Lythrum salicaria* L., purple loosestrife (Lythraceae). Pages 383-390 in P. G. Mason and J. T. Huber, editors. Biological Control Programmes in Canada, 1981-2000. CABI Publishing, Wallingford, UK.
- Mal, T. K., J. Lovett-Doust, and L. Lovett-Doust. 1997. Time-dependent competitive displacement of *Typha angustifolia* by *Lythrum salicaria*. *Oikos* **79**:26-33.
- Malecki, R. A., B. Blossey, S. D. Hight, D. Schroeder, L. T. Kok, and J. R. Coulson. 1993. Biological control of purple loosestrife. *BioScience* **43**:680-686.
- Miller, T. E. and J. Travis. 1996. The evolutionary role of indirect effects in communities. *Ecology* **77**:1329-1335.
- Mothershead, K. and R. J. Marquis. 2000. Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. *Ecology* **81**:30-40.
- Morris, W. F., R. A. Hufbauer, A. A. Agrawal, J. D. Bever, V. A. Borowicz, G. S. Gilbert, J. L. Maron, C. E. Mitchell, I. M. Parker, G. P. Alison, M. E. Torchin, and D. P. Vázquez. 2007. Direct and interactive effects of enemies and mutualists on plant performance: A meta-analysis. *Ecology* **88**:1021-1029.
- Mutikainen, P. and L. F. Delph. 1996. Effects of herbivory on male reproductive success in plants. *Oikos* **75**:353-358.
- Nötzold, R., B. Blossey, and E. Newton. 1998. The influence of below ground herbivory and plant competition on growth and biomass allocation of purple loosestrife. *Oecologia* **113**:82-93.
- Ohashi, K. and T. Yahara. 2001. Behavioral responses of pollinators to variation in floral display size and their influences on the evolution of floral traits. Pages 274-296 in L. Chittka and J. D. Thomson, editors. Cognitive ecology of pollination: Animal behaviour and floral evolution. Cambridge University Press, West Nyack, NY, USA.
- Oleksyn, J., P. Karolewski, M. J. Giertych, R. Zytowskiak, P. B. Reich, and M. G. Tjoelker. 1998. Primary and secondary host plants differ in leaf-level photosynthetic response to herbivory: Evidence from *Alnus* and *Betula* grazed by the alder beetle, *Agelastica alni*. *New Phytologist* **140**:239-249.
- Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* **52**:273-288.
- Poveda, K., I. Steffan-Dewenter, S. Scheu, and T. Tschardt. 2003. Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set. *Oecologia* **135**:601-605.

- Quesada, M., K. Bollman, and A. G. Stephenson. 1995. Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. *Ecology* **76**:437-443.
- Rausher, M. D. and P. Feeny. 1980. Herbivory, plant density, and plant reproductive success: The effect of *Battus philenor* on *Aristolochia reticulata*. *Ecology* **61**:905-917.
- Schat, M. and B. Blossey. 2005. Influence of natural and simulated leaf beetle herbivory on biomass allocation and plant architecture of purple loosestrife (*Lythrum salicaria* L.). *Environmental Entomology* **34**:906-914.
- Schlichting, C. D. and D. A. Levin. 1984. Phenotypic plasticity of annual phlox: Tests of some hypotheses. *American Journal of Botany* **71**:252-260.
- Sharaf, K. E. and M. V. Price. 2004. Does pollination limit tolerance to browsing in *Ipomopsis aggregata*? *Oecologia* **138**:396-404.
- Simberloff, D. 2012. Risks of biological control for conservation purposes. *BioControl* **57**:263-276.
- Stanton, M. L., A. A. Snow, and S. N. Handel. 1986. Floral evolution: Attractiveness to pollinators increases male fitness. *Science* **232**:1625-1627.
- Steets, J. A. and T. L. Ashman. 2004. Herbivory alters the expression of a mixed-mating system. *American Journal of Botany* **91**:1046-1051.
- Strauss, S. Y. 1991. Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. *Ecology* **72**:543-558.
- Strauss, S. Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. *Ecology* **78**:1640-1645.
- Strauss, S. Y., J. K. Conner, and K. P. Lehtilä. 2001. Effects of foliar herbivory by insects on the fitness of *Raphanus raphanistrum*: Damage can increase male fitness. *The American Naturalist* **158**:496-504.
- Strauss, S. Y., J. K. Conner, and S. L. Rush. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: Implications for male and female plant fitness. *The American Naturalist* **147**:1098-1107.
- Strauss, S. Y. and R. E. Irwin. 2004. Ecological and evolutionary consequences of multispecies plant-animal interactions. *Annual Review of Ecology, Evolution, and Systematics* **35**:435-466.
- Stuckey, R. L. 1980. Distributional history of *Lythrum salicaria* (purple loosestrife) in North America. *Bartonia* **47**:3-20.

- Suárez, L. H., W. L. Gonzalez, and E. Gianoli. 2009. Foliar damage modifies floral attractiveness to pollinators in *Alstroemeria exerens*. *Evolutionary Ecology* **23**:545-555.
- Swope, S. M. and I. M. Parker. 2010. Trait-mediated interactions and lifetime fitness of the invasive plant *Centaurea solstitialis*. *Ecology* **91**:2284-2293.
- Swope, S. M. and I. M. Parker. 2012. Complex interactions among biological control agents, pollinators, and an invasive weed: A structural equation modeling approach. *Ecological Applications* **22**:2122-2134.
- Thomson, J. D. 1986. Pollen transport and deposition by bumble bees in *Erythronium*: Influences of floral nectar and bee grooming. *Journal of Ecology* **74**:329-341.
- Thompson, D. W., R. L. Stuckey, and E. B. Thompson. 1987. Spread, impact and control of purple loosestrife (*Lythrum salicaria*) in North American wetlands. Fish and Wildlife Research 2. U.S. Department of the Interior, Fish and Wildlife Service, Washington D.C.
- Treberg, M. and B. Husband. 1999. Relationship between the abundance of *Lythrum salicaria* (purple loosestrife) and plant species richness along the Bar River, Canada. *Wetlands* **19**:118-125.
- Vranjic, J. A. and P. J. Gullan. 1990. The effect of a sap-sucking herbivore, *Eriococcus coriaceus* (Homoptera: Eriococcidae), on seedling growth and architecture in *Eucalyptus blakelyi*. *Oikos* **59**:157-162.
- Weihe, P. E. and R. K. Neely. 1997. The effects of shading on competition between purple loosestrife and broad-leaved cattail. *Aquatic Botany* **59**:127-138.
- Willson, M. F. and N. Burley. 1983. Mate choice in plants: Tactics, mechanisms, and consequences. Princeton University Press, New Jersey, USA.
- Wolfe, L. M. 2002. Why alien invaders succeed: Support for the escape-from-enemy hypothesis. *The American Naturalist* **160**:705-711.
- Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. *Annual Review of Ecology and Systematics* **25**:443-466.
- Zangerl, A. R., J. G. Hamilton, T. J. Miller, A. R. Crofts, K. Oxborough, M. R. Berenbaum, and E. H. de Lucia. 2002. Impact of folivory on photosynthesis is greater than the sum of its holes. *Proceedings of the National Academy of Sciences of the United States of America* **99**:1088-1091.

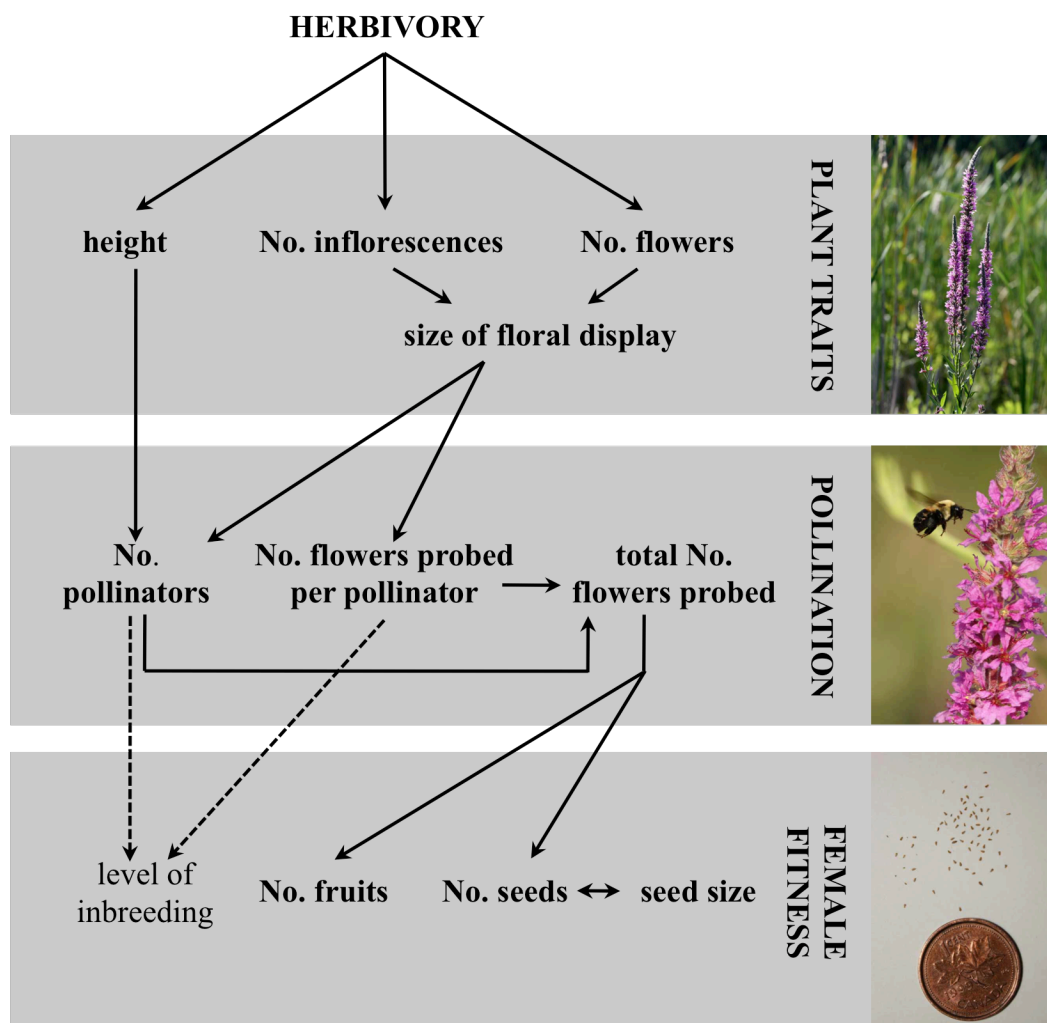


Figure 1.1. Potential indirect effects of herbivory, investigated in this thesis, on the female component of plant fitness via altered plant-pollinator interactions. Dashed lines and non-bold items were not measured. Figure modified from Strauss (1997).

CHAPTER 2: A Study of the Impact of an Introduced Herbivore on Pollinator-Mediated Interactions and Female Fitness in *Lythrum salicaria*

Introduction

Herbivory can affect multiple components of plant fitness, and in doing so may exert selection on flowering plants. In contrast to traditional views of herbivory, which emphasize direct effects on plant fitness, it is becoming increasingly recognized that herbivores can indirectly affect flowering plants by altering interactions between plants and other taxa, such as mutualisms with animal pollinators. By modifying the expression of floral traits, and in turn plant-pollinator interactions, herbivores can influence numerous aspects of sexual reproduction in angiosperms (Figure 1.1), and may impact fitness achieved via female and male function differentially (Strauss 1997, Strauss and Irwin 2004).

Herbivory can affect pollinator-mediated reproduction in angiosperms by altering individual plant traits that function to attract pollinators (reviewed by Strauss 1997, Strauss and Irwin 2004). For example, herbivory has been shown to affect the number of flowers produced (Gronemeyer et al. 1997, Lehtilä and Strauss 1997, Adler et al. 2001, Barber et al. 2011, Barber et al. 2012); flower size (Karban and Strauss 1993, Strauss et al. 1996, Lehtilä and Strauss 1997, Strauss 1997, Lehtilä and Strauss 1999, Mothershead and Marquis 2000, Steets and Ashman 2004, Barber et al. 2011); flowering phenology (Strauss et al. 2001, Poveda et al. 2003, Sharaf and Price 2004, Schat and Blossey 2005, Pellegrino and Musacchio 2006); the size (Lehtilä and Strauss 1999) and quantity of pollen (Frazee and Marquis 1994, Quesada et al. 1995, Strauss et al. 1996, Lehtilä and Strauss 1999); and nectar volume (Krupnick et al. 1999, Halpern et al. 2010) and composition (Adler et al. 2006). Such effects may arise through various mechanisms, including by reducing the resources available

for allocation towards reproduction due to the loss of leaf or root tissues (Mutikainen and Delph 1996, Zamora et al. 1999, Poveda et al. 2003). Floral herbivory may also negatively impact flower appearance (Karban and Strauss 1993) and flower abortion rates (Krupnick and Weis 1998). Furthermore, 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 (Adler et al. 2006). As demonstrated in *Nicotiana quadrivalis* Pursh (Solanaceae), where the response to herbivory (increased nectar production) is dependant on water availability, the direct effects of herbivory on floral traits can vary with aspects of the environment (Halpern et al. 2010). In addition to its influence on individual plant traits, herbivory can alter the density of plant populations and, in doing so, may indirectly affect plant-pollinator interactions and the amount of conspecific pollen received on stigmas (Vázquez and Simberloff 2004).

Following herbivory, the number and/or duration of floral visits by pollinators may decrease (Karban and Strauss 1993, Strauss et al. 1996, Lehtilä and Strauss 1997, Krupnick et al. 1999, Adler et al. 2001, Hambäck 2001, Steets and Ashman 2004, Suárez et al. 2009, Barber et al. 2011, Kessler et al. 2011), increase (Poveda et al. 2003, Barber et al. 2011), or remain unchanged (Sharaf and Price 2004, Hladun and Adler 2009) depending on the way in which herbivory alters the expression of floral traits. For example, feeding by thrips on *Erigeron glaucus* (Asteraceae) flower heads alters the appearance of petals and reduces pollinator visitation (Karban and Strauss 1993). Lehtilä and Strauss (1997) reported that reduced flower production, as a direct result of herbivory, likely explains decreased pollinator visitation to plants that had undergone herbivory treatments. In contrast, increased pollinator visitation may occur when plants respond to herbivory with improved attractiveness to pollinators. For example, root herbivory increased pollinator visitation to

two species, *Sinapis arvensis* (Poveda et al. 2003) and *Cucumis sativus* (Barber et al. 2011). While not measured, the authors suggested that heightened pollinator preference was possibly due to increased nectar production resulting from herbivory in those species (Poveda et al. 2003, Barber et al. 2011).

Altered patterns or rates of pollinator visitation can influence pollen import and export, and can have important consequences for the reproductive component of fitness in flowering plants that rely on pollinators for sexual reproduction (Strauss 1997, Strauss and Irwin 2004). Contrary to previous assumptions, there is not always a positive correlation between male and female fitness in plants, and altered pollinator visitation may affect male fitness despite a lack of apparent effects on female reproductive output, or vice versa (reviewed by Mutikainen and Delph 1996, Strauss 1997). In addition, following herbivory some plant species alter the allocation to male and female function (reviewed by Strauss 1997, also see Lehtilä and Strauss 1999, Thomson et al. 2004). While the mechanisms of altered pollinator visitation are similar (e.g., altered floral display), its effects on male and female fitness are likely to differ, as rates of pollen donation and receipt can have differential effects on fitness accrued through male and female function (Strauss 1997, Krupnick and Weis 1999). According to Bateman's Principle (1948), female fitness should be more resource limited, whereas male fitness should be more heavily impacted by pollinator visitation (i.e., access to mates) (Willson and Burley 1983, Stanton et al. 1986). For example, Bell (1985) noted that female fitness, with respect to the number of ovules fertilized, is expected to be maximized by much fewer pollinator visits than are required to maximize pollen export and, thus, male fitness.

If herbivory leads to a reduction in the number of pollinators, the number of flowers visited per pollinator, and/or the duration of floral visits, then outcrossed pollen export and

receipt may decline, which can affect plant fitness (reviewed by Harder et al. 2001). For instance, the number of pollen grains exported by pollinators, and thus the number available for outcrossing, may decrease if there is a reduction in pollinator visitation (Strauss 1997). This has been demonstrated in *Isomeris arborea* (Capparaceae) where plants subjected to herbivory experienced a 50% reduction in the amount of pollen removed from flowers (Krupnick and Weis 1999). Moreover, reduced pollinator visitation can decrease the amount of outcrossed pollen received by stigmas and therefore the fraction of ovules fertilized (i.e., pollen limitation of female reproduction (Bierzychudek 1981, Burd 1994, Ashman et al. 2004, Knight et al. 2005)). For example, herbivory has been shown to negatively impact pollinator visitation and seed set, thereby decreasing female fitness, in some species (Mothershead and Marquis 2000, Kessler et al. 2011). However, changes in rates of pollinator visitation due to herbivory may not necessarily result in altered fruit and seed set, and these two fitness measures may themselves be limited by resources as a direct affect of herbivory (Hambäck 2001, Poveda et al. 2003, Sharaf and Price 2004, Hladun and Adler 2009).

The number of flowers probed during a foraging bout can have important fitness impacts on plants (de Jong et al. 1993, Barrett and Harder 1996). Modifications to pollinator visitation patterns may have additional effects on female reproductive success by altering offspring quality in species or populations where inbreeding depression is present (Krupnick and Weis 1999). For example, if herbivory leads to an increase in the number of flowers probed during each foraging bout, a response that is likely if damaged plants have more open flowers, the self-fertilization rate will increase (de Jong et al. 1993). Furthermore, increased transfer of pollen to stigmas within the same plant (i.e., pollen discounting) in self-incompatible species is detrimental to male fitness as those gametes will no longer be

available to sire outcrossed seeds (Strauss 1997, Harder and Wilson 1998, Ohashi and Yahara 2001, Harder et al. 2001). Experimental manipulations have shown that as the number of open flowers on a plant increases, so do levels of inbreeding and pollen discounting (Barrett et al. 1994, Harder and Barrett 1995).

In the current study, I investigate the potential for the congeneric beetle species *Galerucella californiensis* L. and *G. pusilla* Duftschmid (Coleoptera: Chrysomelidae), introduced in 1992 as a biological control agent of *Lythrum salicaria*, to alter plant-pollinator interactions and reproductive output. Herbivory by *Galerucella* beetles on *L. salicaria* has been demonstrated to reduce plant height (Blossey and Schat 1997, Schat and Blossey 2005), alter biomass allocation between roots and shoots (Blossey and Schat 1997), reduce biomass (Blossey and Schat 1997), alter plant architecture and increase the number of inflorescences produced (Schat and Blossey 2005), decrease total inflorescence length (Katovich et al. 2001, Schat and Blossey 2005), delay flowering (Schat and Blossey 2005), reduce the number of flowers (Katovich et al. 2001, Landis et al. 2003) and reduce the number of fruits and seeds produced (Katovich et al. 2001). While some of these direct impacts on plant traits may reduce the competitive ability of *L. salicaria* (Malecki et al. 1993, Blossey and Schat 1997, Katovich et al. 2001, Schat and Blossey 2005), modification of a number of these traits has the potential to alter rates of pollinator visitation, which can also affect plant fitness, albeit indirectly (Strauss 1997, Strauss and Irwin 2004). For example, changes in the height of inflorescences may have consequences for pollen transport between undamaged plants and those subjected to herbivory; Levin and Kerster (1973) reported assortative mating in *L. salicaria*, with a significant correlation between the heights of plants visited by individual pollinators. Also, plant height may affect the number and quality of foraging bouts conducted by pollinators. Wolf and Hainsworth (1990) found that, in the species *Ipomopsis aggregata*

(Pursh) V. Grant (scarlet gilia), inflorescence display height positively influenced hummingbird visitation. Changes in floral display size in response to herbivory may also affect pollinator visitation in *L. salicaria*; many studies have demonstrated that pollinator visitation patterns respond to changes in the size of the floral display (Bell 1985, Stanton et al. 1986, Møller and Eriksson 1995, Connor and Rush 1996, Dafni and Kevan 1997, Johnson and Dafni 1998, Ohashi and Yahara 1998, Suárez et al. 2009). Furthermore, subsequent delays in the onset of flowering, following herbivory, may affect overlap with the pollinator community, potentially influencing access to effective pollinators (Sharaf and Price 2004).

The aim of this study was to examine the impacts of *Galerucella* herbivory on pollinator visitation and fruit and seed production in *L. salicaria*. Plants in a natural population were either exposed to herbivory, in ambient (*Galerucella* beetle) and mechanical herbivory treatments, or protected from damage (to the extent possible) in a low herbivory treatment. This was done to determine if direct changes in plant traits, due to *Galerucella* herbivory, result in altered pollinator visitation and differences in fruit and seed production. Since *Galerucella* herbivory has been shown to reduce total inflorescence length (Katovich et al. 2001, Schat and Blossey 2005) and pollinator visitation tends to decrease with the size of the floral display (reviewed by Ohashi and Yahara 2001), I expected that plants in the low herbivory treatment would receive more visits by pollinators and produce more fruits and seeds per fruit than plants exposed to higher levels of damage. This study adds to the relatively limited body of empirical work that investigates the indirect effects of herbivory on plant-pollinator interactions and pairs those observations with measures of fitness, such as reproductive output (Strauss and Irwin 2004). These organisms represent an appropriate study system, as sexual reproduction in *L. salicaria* is dependent on pollinator-mediated interactions (Eckert and Barrett 1992, Eckert 2002) and *Galerucella* herbivory has

previously been shown to alter plant traits (height, plant architecture, floral display size) that may influence rates of pollinator visitation. Additionally, this system provides an opportunity to investigate a potential mechanism (altered pollinator-mediated interactions) through which a biological control agent may impact fitness in an invasive plant species.

Methods

Study species

L. salicaria is a perennial, clonal and heterostylous plant. Multiple stems, which each reach 0.5-2.7 m tall, can arise from common underground rootstock annually (Thompson et al. 1987, Mal et al. 1992, Blossey 1995a). On average, individual *L. salicaria* stems in the study population produced hundreds of flowers during the flowering period, but not all flowers were open at once (J. Russell-Mercier, personal observation). *L. salicaria* depends on animal pollinators, since its primary mode of reproduction is sexual (Eckert and Barrett 1992, Eckert 2002), and seed production is necessary for *L. salicaria* to spread to new habitats (Yakimowski et al. 2005). *L. salicaria*'s red-purple flowers have petals that are ~7-12 mm long (Gleason and Cronquist 1991) and are displayed on numerous spikes (Mal et al. 1992, Schat and Blossey 2005). *L. salicaria*'s flowers are hermaphroditic and individual plants are exclusively either one of three style morphs (short-, mid-, or long-morph) (Mal et al. 1992). Although *L. salicaria* is mostly self-incompatible (due to trimorphic self-incompatibility), some seeds can be produced following self-pollination and there are differences among style morphs in the degree of self-compatibility (both the long- and short-morphs are less self-compatible than the mid-morph) (Darwin 1877, Stout 1923, O'Neil 1994, Ottenbreit and Staniforth 1994, Mal et al. 1999, Colautti et al. 2010). When self-fertilization in *L. salicaria* results in seed production, seedlings exhibit significantly shorter

leaves, significantly fewer leaves, and are significantly shorter than outcrossed seedlings (O'Neil 1994).

Introduced beetles

Due to concerns over the negative effects of *L. salicaria* on North American ecosystems (e.g., its ability to outcompete native flora and/or degrade habitat suitability for some animals), several taxa of beetles, including the congeneric *Galerucella californiensis* and *G. pusilla* beetles, were released in the early 1990s in Canada and the United States for the purpose of biological control (Malecki et al. 1993). The life cycles of *G. californiensis* and *G. pusilla*, which they complete on *L. salicaria*, are similar (Blossey et al. 1994, Blossey 1995b) (Figure 2.1). In Eastern North America adult *Galerucella* beetles emerge between late May to early June (Lindgren et al. 2002), after overwintering near *L. salicaria* in the soil (Lindgren 1999) or leaf litter (Blossey 1995b). Emerging adults feed on leaves and meristems for approximately one week prior to the onset of oviposition (Blossey et al. 1994, Blossey 1995b). Each day individual females can lay up to 10-12 eggs (Blossey 1995b), which are laid on *L. salicaria* over a period of approximately 8-10 weeks, after which the adults die (Malecki et al. 1993, Blossey 1995b). Larvae feed on meristems in addition to leaves and various other tissues (Malecki et al. 1993, Blossey et al. 1994, Hunt-Joshi et al. 2002, Lindgren et al. 2002). Third instar larvae make their way into soil or leaf litter to pupate (Malecki et al. 1993, Blossey et al. 1994, Lindgren et al. 2002, Hunt-Joshi et al. 2004) for a period of 7-10 days (Lindgren 1999). From egg it takes approximately 4-6 weeks to reach the adult stage of the life cycle (Kok et al. 1992, Hunt-Joshi et al. 2004). Prior to returning to the soil or leaf litter for winter diapause, newly emerged adults feed on *L. salicaria* (Hunt-Joshi et al. 2004). Within one year there is normally a single generation, however, in some instances there may be a second generation (Kok et al. 1992, Hunt-Joshi et

al. 2004), which has been observed in my study population (J. Russell-Mercier, personal observation).

Study site

Empirical work 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. The study population covered an area of approximately 10,000 m², with approximately 3.7 ± 6.1 (SD) stems per m² (averaged over 60 random 0.38 m² plots). Species co-occurring with *L. salicaria* at the study site included *Decodon verticillatus* (L.) Elliott. (Lythraceae), *Nuphar variegata* Durand. (Nymphaeaceae), *Nymphaea odorata* Aiton. (Nymphaeaceae), *Scutellaria galericulata* L. (Lamiaceae), *Hydrocharis morsus-ranae* L. (Hydrocharitaceae), *Galium palustre* L. (Rubiaceae), *Campanula aparinoides* Pursh. (Campanulaceae), *Impatiens capensis* Meerb. (Balsaminaceae), *Asclepias incarnata* L. (Asclepiadaceae), *Solanum dulcamara* L. (Solanaceae), *Mimulus* sp. (Scrophulariaceae), *Sagittaria* sp. (Alismataceae), *Typha latifolia* L. (Typhaceae), *Calla palustris* L. (Araceae), various grasses, and sedges.

Herbivory treatments

105 *L. salicaria* stems were haphazardly selected throughout Cow Island Marsh in the second week of June 2011. Stems were selected to be at least 1 m apart to reduce the probability of including more than one stem from a given individual (following Haldane 1936, Eckert and Barrett 1992, Hermann et al. 1999). All stems included in the study population were selected to be approximately the same height, with no visible meristem damage. Moreover, included stems were visually inspected for *Galerucella*, and all detected adults, larvae and egg masses were physically removed. Each stem was then covered with a herbivore-exclusion sleeve, made of white no-see-um netting (Rose e Dee Ltd.) (Figure 2.2),

and randomly assigned to one of three treatments ($n = 35$ per treatment): low herbivory, ambient herbivory, or mechanical herbivory.

Herbivory treatments began during the third week of June, 2011. In the low herbivory treatment, plants were inspected at least once a week for the presence of *Galerucella* beetles, to ensure minimal feeding damage (i.e., detected *Galerucella* beetles were removed), until pollinator observations were conducted. For the ambient herbivory treatment, seven *Galerucella* larvae, collected from two different *L. salicaria* populations, were added to each stem before the placement of the cover. The larvae were collected from the study population at Cow Island Marsh and from a second population at Indian Lake, located approximately 1.5 km away from the study population. The larvae were transported in Petri dishes with *L. salicaria* leaves and placed on ambient herbivory treatment plants within a few hours of being collected. Seven larvae were chosen because it was the average number of larvae counted on a haphazard sample of plants in the study population ($n = 16$). When placing the larvae on ambient treatment plants I ensured that two larvae were added to the apical meristem. For the mechanical treatment, once a week, for a period of three weeks, the apical meristem of each plant was clipped with scissors and a leaf disk was taken from each leaf that was large enough to have a leaf disk removed without cutting the leaf in two. At the end of three weeks of applying mechanical damage, the average percent leaf damage (visual estimate) of mechanical treatment plants was similar to that of haphazardly inspected plants ($n = 17$) that were intermingled with the study population. Due to the loss (by suspected deer trampling) of two low herbivory treatment plants early in the study, two replacement plants were haphazardly chosen, cleaned of larvae, and covered with herbivore-exclusion sleeves.

Herbivore-exclusion sleeves were removed from all plants in the study population on July 8 and 9, soon after the start of flowering, in order to perform the pollinator observations

and allow plants to set seed. All herbivore-exclusion sleeves were removed at the same time in order to reduce the potentially confounding effects of covering plants for different lengths of time. A second generation of *Galerucella* beetles was observed after the herbivore-exclusion sleeves were removed. Plants in the study population were checked at least once a week, and all detected eggs, larvae, and adults were removed, until pollinator observations began. Beetles, eggs and larvae were removed during this period to limit pre-flowering herbivory on the low and mechanical treatments and to minimize variation in herbivore damage as much as possible. Occasionally Japanese beetles (*Popillia japonica*) were observed feeding on plants in the study population.

Pollinator observations

Pollinator observations were conducted from July 26 through August 5, 2011, on sunny/mainly sunny days without strong wind, between ~ 9:00 - 17:00 h (following King and Sargent 2012). Each 30-minute observation period consisted of one observer recording the identity and foraging behavior of pollinators on a single plant using a hand-held digital voice recorder. Pollinators from five taxonomic groups (*Bombus* spp., solitary bees, *Apis mellifera* (honeybees), Syrphid flies, and wasps) were observed visiting focal plants during pollinator observations and, during foraging bouts, pollinators were classified to one of these groups (following Da Silva et al. In Press). A visit was defined as a pollinator probing a flower and contacting the stigma and/or anthers (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, during which time a total of 1164 foraging bouts and 6272 visits were recorded. Prior to each observation period the following information was recorded: the number of open flowers per inflorescence on the target plant; the height of the tallest inflorescence on the target plant (cm); the number of

non-target *L. salicaria* and *Decodon verticillatus* flowering stems, and the number and identity of co-flowering plants within a 0.38 m² circular plot around the target plant.

Due to the delayed onset of flowering of some plants, and damage (browsing and/or trampling by deer, etc.) to other plants, the sample size was reduced from 105 to 83 plants (low herbivory: $n = 26$, ambient herbivory: $n = 29$, mechanical herbivory: $n = 28$). 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 towards any treatment with respect to observer ($\chi^2 = 8.677$, $df = 12$, $p = 0.7302$), date ($\chi^2 = 13.669$, $df = 20$, $p = 0.8469$), or time of day ($\chi^2 = 16.467$, $df = 14$, $p = 0.2857$). There were no significant differences in the densities of non-target *L. salicaria* ($F = 0.7234$, $df = 2$, $p = 0.4883$), *D. verticillatus* ($F = 2.0540$, $df = 2$, $p = 0.1392$), or combined total of the two species' ($F = 0.9931$, $df = 2$, $p = 0.3750$) flowering stems surrounding each observed plant.

Fruit and seed production

Fruit was collected from all plants in the fall of 2011 after the maturation period, and then stored in coin envelopes in a lab at the University of Ottawa. The height (cm) of the tallest fruiting stalk on each plant was measured prior to fruit removal and the total length of fruiting stalks was measured with a digital caliper (Mitutoyo Inc.). 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, implemented in MATLAB (The Mathworks Inc. 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 (low: $n = 24$, ambient: $n =$

27, mechanical: $n = 29$) due to loss of additional individuals in the weeks between pollinator observations and fruits collection (due to browsing and/or trampling by deer, etc.).

Statistical analyses

Individual plants were used as the unit of replication for all analyses. Pollinator visitation, fruit and seed production were analyzed as plant averages using one-way ANOVA, weighted by the number of observations per individual plant, and with treatment as a fixed effect. All observation periods were used to calculate the average number of foraging bouts and the average number of flowers probed per plant per 30-minute period. However, because analyses pertaining to the number of flowers probed per foraging bout and the average number of switches among inflorescence per foraging bout were specific to the behaviour of pollinators during individual foraging bouts, observation periods with 0 pollinator visitors were necessarily excluded from these particular (per foraging bout) analyses. Seed counts were averaged for each plant. When necessary, data was transformed to meet the normality assumption of parametric statistics (Quinn and Keough 2002). In some instances Kruskal-Wallis tests were used to compare groups when data failed to meet the normality assumption of parametric statistics. However, since ANOVA is robust against violations of normality (Quinn and Keough 2002), and Kruskal-Wallis and ANOVA provided quantitatively similar results, only ANOVA are reported. Welch's ANOVA was used when data were heteroscedastic. Post-hoc testing was conducted using Tukey's HSD test when significant differences were detected among treatments.

Regression analyses were used to examine relationships between plant traits that could be categorized as continuous (number of flowers, number of inflorescences, and height) and aspects of pollinator visitation patterns. Regression analyses were used because

ANCOVA (with plant trait values as covariates) was not deemed to be an appropriate test, as the covariates listed above were all confounded by treatment.

ANCOVA, weighted by the number of pollinator observation periods per plant, was used to investigate the effects of the surrounding plant community on herbivore-mediated changes in each measured aspect of pollinator visitation. *L. salicaria* and *D. verticillatus* were the dominant species surrounding the focal plants and their densities were therefore the only species included in ANCOVA. For each ANCOVA, the density of non-target *L. salicaria* and *D. verticillatus* flowering stems within the 0.38 m² plot was used as a covariate, and treatment was a fixed effect. All analyses were performed in JMP (SAS 2012).

Results

Direct effects of herbivory

At the onset of the experimental period there were no significant differences in average plant height (cm) among treatments ($F = 1.2338$, $df = 2$, $p = 0.2969$). However, there were significant differences in mean height (cm) among treatments during pollinator observations ($F = 6.5686$, $df = 2$, $p = 0.0030$). This difference was still detectable when fruits were harvested at the end of the growing season ($F = 3.3931$, $df = 2$, $p = 0.0387$). The mean height of plants in the ambient herbivory treatment was significantly lower than in either the low or mechanical herbivory treatments during pollinator observations. At the end of the growing season the mean height of plants in the ambient herbivory treatment was significantly lower than plants in the low herbivory treatment (Figure 2.3). Mean plant height did not significantly differ between the low and mechanical herbivory treatments at any measured point in time.

The number of inflorescences, as measured at the start of each pollinator observation period, differed significantly among treatments ($F = 11.6486$, $df = 2$, $p < 0.0001$). The mean number of inflorescences in the low herbivory treatment was significantly less than for the ambient or mechanical herbivory treatments, which were not significantly different from each other (Figure 2.4). Treatment also had a significant effect on the number of fruiting stalks produced per plant ($F = 5.6617$, $df = 2$, $p = 0.0051$); low herbivory plants produced significantly fewer fruiting stalks than plants in the mechanical herbivory treatment (Figure 2.4).

There was a significant effect of treatment on the date of first flower ($F = 6.1884$, $df = 2$, $p = 0.0033$) and the mean number of open flowers during the period of pollinator observations ($F = 5.1402$, $df = 2$, $p = 0.0079$). The onset of flowering was significantly earlier in the low herbivory treatment, by approximately 6 days on average, than for either the ambient or mechanical herbivory treatments (Figure 2.5A). During pollinator observations, plants in the ambient and mechanical herbivory treatments tended to have more open flowers than plants in the low herbivory treatment, but only the difference between the mechanical and low herbivory treatments was significant (Figure 2.5B).

Pollinator community

Bombus spp. comprised 89.0%, 85.4% and 90.0% of the total number of pollinators that visited low, ambient and mechanical herbivory treatment plants, respectively. Other pollinator taxa 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 pollinator community (0.4% and 0.6%, respectively, of visitors to the low and mechanical herbivory treatments), did not visit the

ambient herbivory treatment plants, and were only recorded on four out of eleven observation days. The remaining 0.4 – 0.6% of pollinators were unidentified (Figure 2.6).

Pollinator visitation

There were no significant differences among treatments in the number of foraging bouts (defined as a pollinator visiting ≥ 1 flowers on a focal plant) per 30 minutes ($F = 2.1676$, $df = 2$, $p = 0.1211$). However, there was a marginally significant difference among the three treatments in the average number of flowers probed per observation period ($F = 2.9206$, $df = 2$, $p = 0.0597$). In general, plants in the ambient and mechanical herbivory treatments had more flowers probed per observation period than plants in the low herbivory treatment (Figure 2.7A). In addition, there was a marginally significant difference among treatments with respect to the number of flowers probed per foraging bout ($F = 3.0150$, $df = 2$, $p = 0.0554$). On average, the number of flowers probed was lowest in the low herbivory treatment and highest in the mechanical herbivory treatment (Figure 2.7B). The herbivory treatment had a significant effect on the mean number of switches among inflorescences per foraging bout ($F = 9.1939$, $df = 2$, $p = 0.0002$). Specifically, pollinators made significantly more switches among inflorescences in the ambient and mechanical herbivory treatments, relative to the low herbivory treatment. There were no significant differences among treatments in the average number of flowers probed per inflorescence visited ($F = 2.2546$, $df = 2$, $p = 0.1124$).

When analyses were restricted to visits by *Bombus* species only, results were similar as when all pollinator types were pooled. However, when *Bombus* species were exclusively considered in the analyses of aspects of pollinator visitation, there was a significant effect of treatment on the number of flowers probed per 30 minutes, with plants in the low herbivory

treatment receiving significantly fewer flowers probed than plants in the mechanical herbivory treatment ($F = 3.4481$, $df = 2$, $p = 0.0366$) (Figure 2.7A).

It is unlikely that differences in pollinator visitation were due to the date or time of day of the observations. There were no clear patterns for date or time of day effects across plants that had 4 or 5 (the maximum number of observation periods per plant) observation periods. Moreover, there were no significant differences in the date ($\chi^2 = 13.669$, $df = 20$, $p = 0.8469$) or time of day ($\chi^2 = 16.467$, $df = 14$, $p = 0.2857$) of observation periods across treatments.

Effects of plant characteristics on pollinator visitation

There was a significant positive linear relationship among three key plant characteristics (plant height, the number of flowers per plant and the number of inflorescences per plant) and various aspects of the pollinator visitation data (Table 2.1). The number of foraging bouts, the number of flowers probed (per 30 minutes and per foraging bout), and the number of switches among inflorescences all increased as each of the aforementioned plant traits increased in value. The average number of open flowers per plant during pollinator observations explained the greatest amount of variation in the number of foraging bouts and flowers probed. The average number of inflorescences per plant explained the largest amount of variation in the number of switches among inflorescences per foraging bout (Table 2.1). Collinearity existed among these traits as there were significant positive linear relationships between the number of flowers (square root transformed to achieve normality of residuals) and plant height (R^2 adj = 0.26, $F_{(1,79)} = 28.39$, $p < 0.0001$), the number of flowers and the number of inflorescences (R^2 adj = 0.77, $F_{(1,81)} = 283.83$, $p < 0.0001$), and the number of inflorescences (square root transformed to achieve normality of residuals) and plant height (R^2 adj = 0.13, $F_{(1,79)} = 13.39$, $p = 0.0005$).

There is no reason to expect that style morph length affects pollinator behaviour. Furthermore, style morphs were evenly distributed among each treatment as indicated by a Pearson χ^2 test ($\chi^2 = 7.156$, $df = 4$, $p = 0.1279$). Hence, morph was not included as a factor in the analyses.

Relationships between plant community and pollination

The number of foraging bouts and the number of flowers probed per 30-minutes increased positively with the density of surrounding *L. salicaria* and *D. verticillatus* flowering stems (Tables 2.2 & 2.3). However, there was no statistically significant relationship between the average number of flowers probed per foraging bout and the density of surrounding *L. salicaria* and *D. verticillatus* flowering stems (Table 2.4). The herbivory treatment had a significant effect on the number of flowers visited per foraging bout when the densities of *L. salicaria* and *D. verticillatus* flowering stems surrounding focal plants were considered in the analysis (Table 2.4). Specifically, during individual foraging bouts, pollinators probed significantly more flowers in the mechanical herbivory treatment than in the low herbivory treatment.

It is unlikely that differences among treatments in terms of pollinator visitation can be attributed to the floral density of neighbouring plants. There were no significant differences in the number of non-focal *L. salicaria* and *D. verticillatus* flowering stems surrounding focal plants in each treatment during pollinator observations ($F = 0.9931$, $df = 2$, $p = 0.3750$). Also, the p -value of herbivory treatment changes little between ANOVA (treatment as fixed effect) and ANCOVA (treatment as a fixed effect; number of surrounding *L. salicaria* and *D. verticillatus* flowering stems as the covariate) models.

Fruit and seed production

Treatment did not have a significant effect on the total length of the harvested fruiting stalks ($F = 1.6090$, $df = 2$, $p = 0.2068$). No statistically significant differences were found among treatments in the mean number of fruits per plant ($F = 1.8691$, $df = 2$, $p = 0.1612$), the mean number of seeds per five randomly chosen fruits ($F = 0.2921$, $df = 2$, $p = 0.7475$), or the mean number of seeds per plant ($F = 1.5623$, $df = 2$, $p = 0.2162$). Because plants produced different numbers of flowers, I also tested the number of fruits and seeds per length of fruiting stalk, which should correlate with inflorescence length and the total number of flowers produced per plant. However, as this did not change the results (i.e., there were no significant differences among treatments in the number of fruits or seeds per total length of fruiting stalks), I report only the number of fruits and seeds. There were significant positive relationships between plant height and the number of fruits per plant (R^2 adj = 0.39, $F_{(1,79)} = 52.0970$, $p < 0.0001$), the number of seeds per fruit (R^2 adj = 0.10, $F_{(1,79)} = 9.4779$, $p = 0.0029$), and the number of seeds per plant (R^2 adj = 0.46, $F_{(1,79)} = 69.5101$, $p < 0.0001$).

Discussion

Herbivory, both mechanically and by *Galerucella* beetles, directly affected several *L. salicaria* traits. Plant height, a potential measure of resource acquisition (Mitchell 1994), and the timing of the onset of flowering, which may influence overlap with the pollinator community (Sharaf and Price 2004), were negatively influenced by the ambient and mechanical herbivory treatments. However, the ambient and mechanical herbivory treatments had a positive impact on the number of open flowers (as measured during pollinator observations) and inflorescences. Apex removal has previously been shown to

increase the number of *L. salicaria* shoot meristems (Venecz and Aarssen 1998, Schat and Blossey 2005), which may in turn develop into inflorescences.

Interactions between *L. salicaria* plants and pollinators were affected by the herbivory treatments, as evidenced by an increase in the number of flowers probed (per 30-minutes and per foraging bout) in the ambient and mechanical herbivory treatments, compared to the low herbivory treatment. Other authors have also reported heightened pollinator visitation to plants damaged by herbivores. In two separate studies, increased nectar production was suggested as a potential cause of increased pollinator preference towards plants exposed to root herbivory (Poveda et al. 2003, Barber et al. 2011). In the current experiment, the observed differences in pollinator visitation were likely mediated primarily by a difference in the number of open flowers among treatments as, of the plant traits measured, flower number explains the greatest amount of variation in the number of flowers probed per 30-minutes and per foraging bout. This result is consistent with previous empirical work demonstrating a positive relationship between the number of open flowers on a plant and the number of flowers visited by each pollinator (reviewed by Ohashi and Yahara 2001). Changes in plant architecture (the number of inflorescences), as a direct response to herbivory, contributed to differences in the number of switches among inflorescences and, in turn, the number of flowers probed in my study. There was no significant difference among treatments in the mean number of flowers probed per inflorescence visited, but there were significant differences in the number of switches among inflorescences per foraging bout. Furthermore, the number of inflorescences was the strongest predictor of the number of switches among inflorescences per foraging bout.

The number of foraging bouts and the number of flowers probed per 30-minutes increased with the density of co-flowering *L. salicaria* and *D. verticillatus* flowering stems

surrounding each focal plant. This suggests that the density of the co-flowering plant community may be important for pollinator attraction and is consistent with previous studies showing that density can affect pollinator attraction (Feinsinger et al. 1986, Klinkhamer and de Jong 1990, Kunin 1997) and/or plant reproductive success (Knight 2004, Vázquez and Simberloff 2004), possibly because pollinators can minimize energy expenditure by making shorter inter-flower flights in patches with high densities of flowers (Klinkhamer and de Jong 1990). However, in the current experiment, there was no relationship between the number of flowers probed per foraging bout and the density of surrounding *L. salicaria* and *D. verticillatus* flowering stems, suggesting that once a pollinator initiated a foraging bout on a *L. salicaria* plant, the density of co-flowering species did not influence the number of flowers visited by that pollinator.

Despite significant differences in pollinator visitation among treatments, a difference in fruit and seed production among treatments was not detected. This suggests that, relative to the low herbivory treatment, female reproduction in the ambient and mechanical herbivory treatments was not limited by resources, as a direct effect of herbivory, or by pollinator visitation, as an indirect effect of herbivory. Poveda et al. (2003) also reported significantly increased pollinator visitation, as an indirect effect of herbivory, without subsequent significant differences in seed set. Furthermore, increased pollinator visitation does not always reduce the pollen limitation of seed set. For example, in cucumber (*Cucumis sativus*) plants that experienced root herbivory, Barber et al. (2011) reported both increased honeybee visitation and heightened pollen limitation.

Several factors may have contributed to the lack of difference in fruit and/or seed production in the current study. First, if baseline levels of pollinator visitation were such that all plants received enough pollen to fertilize nearly all ovules, then higher levels of pollinator

visitation in the mechanical and ambient herbivory treatments would not result in greater numbers of fruits or seeds produced. Second, although depleted resources due to herbivory can restrict ovule production, plants in the ambient and mechanical herbivory treatments may have allotted a greater proportion of resources to ovule production in order to maintain fitness achieved via female function (Lehtilä and Strauss 1999). Third, fruit abortion rates may have been higher in the ambient and mechanical herbivory treatments, as fruit abortion rates are significantly higher in branches subjected to defoliation, relative to non-defoliated branches in some species (Stephenson 1980, Niesenbaum 1996). Higher fruit abortion rates may arise from the resource limitation of fruit and seed production resulting from differences in resource acquisition and/or allocation (Stephenson 1981, Willson and Burley 1983, Niesenbaum 1996). However, it is unlikely that fruit abortion rates differed among treatments because treatment did not have a significant effect on the number of fruits per length of fruiting stalk (proxy for proportion of fruit per flower). Fourth, an increased sample size for seed counts may have raised the power to detect a significant effect of treatment, if one existed. Seeds were counted from 5 fruits per plant, which represents on average ~1.3% of the fruits per plant (min = ~0.4% of fruits, max = ~38.5% of fruits). There were also error rates associated with the seed counting program, but these were low as determined by a test of 2307 seeds (false negative rate = ~2.9%, false positive rate = ~1.7%) (L. Remmler, personal communication) and did not significantly differ among treatments. It is unlikely that an increased sample size would have detected significant differences among treatments as the p -value was very high, suggesting that the null hypothesis (no difference in the number of seeds per fruit among treatments) was not very likely to be false. Furthermore, other authors have reported no effect of *Galerucella* herbivory on the number of *L. salicaria* seeds per fruit (Katovich et al. 2001).

Differences among treatments in the number of flowers probed, both per 30-minutes and per foraging bout, could have important effects on plant fitness by altering gene flow (via pollen transfer and receipt) (Harder et al. 2001, Ohashi and Yahara 2001). The probability of transferring self-pollen to stigmas rises with the number of flowers visited during a given foraging bout (de Jong et al. 1993). Thus, female reproductive success may have been negatively impacted as *L. salicaria* is partially self-compatible at low levels (Darwin 1877, Stout 1923, Ottenbreit and Staniforth 1994, O'Neil 1994, Mal et al. 1999, Colautti et al. 2010) and this species suffers from inbreeding depression; seeds produced by self-pollination are of significantly reduced quality with respect to several growth parameters (leaf size, number of leaves, and height – see O'Neil 1994). Furthermore, the number of seeds produced per *L. salicaria* fruit is decreased by the deposition of self-pollen on stigmas prior to receipt of outcrossed pollen (Nicholls 1987). Although there were no significant differences in the number of seeds per fruit among treatments in the current study, it is possible that if the number of flowers probed per foraging bout (and self-pollen deposition) was higher, then herbivory may have indirectly reduced seed set.

Male fitness, which was not measured, may have been affected in distinct ways by differences in the number of flowers probed per 30-minutes and per foraging bout, which respectively influence both pollen export and pollen discounting (Ohashi and Yahara 2001). For example, Rush et al. (1995) reported a significant positive linear relationship between the number of visits conducted by bees and the amount of pollen exported from individual flowers. Hence, increased pollinator visitation may have increased male fitness in the ambient and mechanical herbivory treatments by increasing total pollen export and the number of seeds sired per plant (Harder et al. 2001). 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*), following initial visits, additional visits by pollinators to individual flowers only had an effect on the amount of pollen removed in mid-level anthers (Harder and Barrett 1993).

An increase in the number of flowers probed per foraging bout, as observed in the current study, may negatively affect male fitness by reducing the proportion of pollen that sire seeds; as the length of a given foraging bout increases, the probability that pollen grains are lost also increases (Biernaskie and Cartar 2004). For example, pollen may be groomed off of pollinators' bodies, buried under subsequently collected grains, or transferred within the same plant (reviewed by Harder et al. 2001, Biernaskie and Cartar 2004). Pollen transferred to stigmas of flowers on the same plant, defined as pollen discounting, in a self-incompatible species such as *L. salicaria*, is particularly detrimental to male fitness as those pollen grains are no longer available for selfing or outcrossing (Harder and Wilson 1998, Harder et al. 2001). Thus, the increased number of flowers probed per foraging bout in the ambient herbivory and mechanical herbivory treatments, relative to the low herbivory treatment, may have reduced male fitness by increasing the extent of pollen discounting. This is likely, as plants in the mechanical and ambient herbivory treatments had more open flowers (during pollinator observations) than plants in the low herbivory treatment and levels of pollen discounting increases with the number of open flowers (Barrett et al. 1994, Harder and Barrett 1995).

The ambient and mechanical herbivory treatments may have had additional unknown effects directly on male fitness. For example, the damage imposed on plants may have decreased the number of pollen grains per flower (Frazee and Marquis 1994, Strauss et al. 1996) and/or reduced the quality of pollen (Quesada et al. 1995, Mutikainen and Delph 1996) from damaged plants, relative to undamaged plants (also reviewed by Delph et al.

1997). For example, herbivory can decrease rates of pollen tube growth from males that experience herbivory, thereby making them less likely to fertilize ovules when competing against pollen from undamaged males (Mutikainen and Delph 1996).

The results presented here contradict a previous conclusion that meristem damage across a plant, caused by *Galerucella* larval feeding, at any intensity will reduce flower production and, in turn, the total number of fruits and seeds produced in *L. salicaria* (Katovich et al. 2001). However, although I found generally positive effects of ambient and mechanical herbivory on *L. salicaria* rates of pollinator visitation, and no effect on seed or fruit production, this does not imply that *Galerucella* are an ineffective biological control for *L. salicaria*. Rather, as plant responses can vary under different extents of damage (Doak 1992), the levels of foliar and meristem damage imposed on plants during the experiment may have been insufficient to reduce fruit or seed production. Moreover, it may be possible that the effects of herbivory compound over time, such that multiple years of herbivory reduce flowering, or that effects of herbivory are present even in the seasons following feeding damage (Strauss 1991, Karban and Strauss 1993). Thus, if the extent of herbivory applied in the current experiment was greater within the season and/or if herbivory treatments were applied over multiple years, then fruit and seed production may have been reduced as it has been shown that *Galerucella* herbivory can reduce or prevent flower production and, in turn, reproductive output (Lindgren 1999, Landis et al. 2003, Katovich et al. 2001). Abiotic environmental conditions may have also contributed to the observed responses of *L. salicaria* to the ambient and mechanical herbivory treatments (e.g., Halpern et al. 2010, Brody and Irwin 2012). Finally, it is not known if the herbivory treatments facilitated native flora by reducing other aspects of *L. salicaria*'s competitive ability, such as changing light or nutrient availability. For example, feeding by *Galerucella* beetles upon *L.*

salicaria can result in more light penetrating through the canopy that may be accessible to other plants (Hunt-Joshi et al. 2004).

There were no detectable differences between the mechanical and ambient herbivory treatments, with the exception of inflorescence height. This indicates that these two treatments affected the plants in a similar fashion, contrasting Schat and Blossey (2005). It is likely that the difference is owing to the fact that Schat and Blossey (2005) did not damage meristems. Venecz and Aarssen (1998) studied the effects of removing *L. salicaria* shoot apices and found that clipping of all apices prior to flowering delayed the onset of flowering by approximately one month, significantly reduced plant height, and significantly increased the number of shoots. Their pre-flowering clipping treatment did not significantly affect the number of inflorescences produced, as was found in my experiment, but the level of apex removal (all apices clipped) was more severe than any of my treatments. Although I did not manipulate larval and adult *Galerucella* herbivory independently, my experimental results, taken in conjunction with the work of Schat and Blossey (2005) and Venecz and Aarssen (1998), suggest that many of the effects of *Galerucella* herbivory, both direct and indirect, are mediated by larval feeding on meristems instigating the release of apical dominance.

Conclusions

The results of this experiment indicate that *Galerucella* beetle herbivory directly affects *L. salicaria* traits, which is consistent with previous reports (Blossey and Schat 1997, Katovich et al. 2001, Landis et al. 2003, Schat and Blossey 2005). The mechanical and ambient herbivory treatments had similar effects on plant traits, which suggests that plant responses to *Galerucella* herbivory are induced by physical leaf area removal and meristem damage, rather than by chemical cues received from the beetles (see Van Zandt and Agrawal

2004, Voelckel and Baldwin 2004). Direct effects of *Galerucella* herbivory on *L. salicaria* include reduced biomass (Blossey and Schat 1997), reduced height (Blossey and Schat 1997, Schat and Blossey 2005), and altered biomass allocation (Blossey and Schat 1997) in addition to reduced flower and, in turn, fruit production (Katovich et al. 2001). Moreover, the work presented here demonstrates that herbivory by *Galerucella* beetles has the potential to indirectly affect pollinator-mediated fitness in *L. salicaria*, as demonstrated by increased pollinator visitation to the ambient and mechanical herbivory treatments compared to the low herbivory treatment. The effect of increased pollinator visitation was likely mediated by increased inflorescence and flower production following herbivory. I did not detect an effect of herbivory on fruit and seed production. However, additional effects of increased pollinator visitation on *L. salicaria* may be present, such as reduced male (i.e., increased pollen discounting) or female (i.e., increased inbreeding) reproductive success. Thus, this study highlights the importance of considering and investigating multispecies interactions when attempting to understand or predict the effects of biological controls on the invasive species that they are intended to control (also see Swope and Parker 2012). Furthermore, this study adds to the growing body of literature on multispecies interactions and is one of relatively few experiments to demonstrate increased pollinator preference towards plants subjected to herbivory (but see Poveda et al. 2003, Poveda et al. 2005, Barber et al. 2011).

References

- Adler, L. S., R. Karban, and S. Y. Strauss. 2001. Direct and indirect effects of alkaloids on plant fitness via herbivory and pollination. *Ecology* **82**:2032-2044.
- Adler, L. S., M. Wink, M. Distl, and A. J. Lentz. 2006. Leaf herbivory and nutrients increase nectar alkaloids. *Ecology Letters* **9**:960-967.
- Ashman, T. L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, S. J. Mazer, R. J. Mitchell, M. T. Morgan, and W. G. Wilson. 2004. Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology* **85**:2408-2421.
- Barber, N. A., L. S. Adler, and H. L. Bernardo. 2011. Effects of above- and belowground herbivory on growth, pollination, and reproduction in cucumber. *Oecologia* **165**:377-386.
- Barber, N. A., L. S. Adler, N. Theis, R. V. Hazzard, and E. T. Kiers. 2012. Herbivory reduces plant interactions with above- and belowground antagonists and mutualists. *Ecology* **93**:1560-1570.
- Barrett, S. C. H. 2002. The evolution of plant sexual diversity. *Nature Reviews Genetics* **3**:274-284.
- Barrett, S. C. H. and L. D. Harder. 1996. Ecology and evolution of plant mating. *Trends in Ecology & Evolution* **11**:73-79.
- Barrett, S. C. H., L. D. Harder, and W. W. Cole. 1994. Effects of flower number and position on self-fertilization in experimental populations of *Eichhornia paniculata* (Pontederiaceae). *Functional Ecology* **8**:526-535.
- Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* **2**:349-368.
- Bell, G. 1985. On the function of flowers. *Proceedings of the Royal Society of London B: Biological Sciences* **224**:223-265.
- Biernaskie, J. M. and R. V. Cartar. 2004. Variation in rate of nectar production depends on floral display size: A pollinator manipulation hypothesis. *Functional Ecology* **18**:125-129.
- Bierzychudek, P. 1981. Pollinator limitation of plant reproductive effort. *The American Naturalist* **117**:838-840.
- Blossey, B. 1995a. A comparison of various approaches for evaluating potential biological control agents using insects on *Lythrum salicaria*. *Biological Control* **5**:113-122.

- Blossey, B. 1995b. Coexistence of two leaf-beetles in the same fundamental niche. Distribution, adult phenology and oviposition. *Oikos* **74**:225-234.
- Blossey, B. and M. Schat. 1997. Performance of *Galerucella californiensis* (Coleoptera: Chrysomelidae) on different North American populations of purple loosestrife. *Environmental Entomology* **26**:439-445.
- Blossey, B., D. Schroeder, S. D. Hight, and R. A. Malecki. 1994. Host specificity and environmental impact of two leaf beetles (*Galerucella californiensis* and *G. pusilla*) for biological control of purple loosestrife (*Lythrum salicaria*). *Weed Science* **42**:134-140.
- Brody, A. K. and R. E. Irwin. 2012. When resources don't rescue: Flowering phenology and species interactions affect compensation to herbivory in *Ipomopsis aggregata*. *Oikos* **121**:1424-1434.
- Burd, M. 1994. Bateman principle and plant reproduction: The role of pollen limitation in fruit and seed set. *Botanical Review* **60**:83-139.
- Conner, J. K. and S. Rush. 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia* **105**:509-516.
- Colautti, R. I., N. A. White, and S. C. H. Barrett. 2010. Variation of self-incompatibility within invasive populations of purple loosestrife (*Lythrum salicaria* L.) from Eastern North America. *International Journal of Plant Sciences* **171**:158-166.
- Dafni, A. and P. G. Kevan. 1997. Flower size and shape: Implications in pollination. *Israel Journal of Plant Sciences* **45**:201-212.
- Darwin, C. R. 1877. The different forms of flowers on plants of the same species. J Murray, London.
- Da Silva, E. M., V. M. King, J. M. Russell-Mercier, and R. D. Sargent. In Press. Evidence for pollen limitation of a native plant in invaded communities. *Oecologia*.
- de Jong, T. J., N. M. Waser, and P. G. L. Klinkhamer. 1993. Geitonogamy: The neglected side of selfing. *Trends in Ecology & Evolution* **8**:321-325.
- Delph, L. F., M. H. Johannsson, and A. G. Stephenson. 1997. How environmental factors affect pollen performance: Ecological and evolutionary perspectives. *Ecology* **78**:1632-1639.
- Doak, D. F. 1992. Lifetime impacts of herbivory for a perennial plant. *Ecology* **73**:2086-2099.
- Eckert, C. G. 2002. The loss of sex in clonal plants. *Evolutionary Ecology* **15**:501-520.

- Eckert, C. G. and S. C. H. Barrett. 1992. Stochastic loss of style morphs from populations of tristylous *Lythrum salicaria* and *Decodon verticillatus* (Lythraceae). *Evolution* **46**:1014-1029.
- Feinsinger, P., K. G. Murray, K. Sharon, and W. H. Busby. 1986. Floral neighborhood and pollination success in four hummingbird-pollinated cloud forest plant species. *Ecology* **67**:449-464.
- Frazer, J. E. and R. J. Marquis. 1994. Environmental contribution to floral trait variation in *Chamaecrista fasciculata* (Fabaceae, Caesalpinoideae). *American Journal of Botany* **81**:206-215.
- Gleason, H. A. and A. Cronquist. 1991. Manual of vascular plants of northeastern United States and adjacent Canada. 2nd edition. The New York Botanical Garden, Bronx, New York.
- Gronemeyer, P. A., B. J. Dilger, J. L. Bouzat, and K. N. Paige. 1997. The effects of herbivory on paternal fitness in scarlet gilia: Better moms also make better pops. *The American Naturalist* **150**:592-602.
- Haldane, J. B. S. 1936. Some natural populations of *Lythrum salicaria*. *Journal of Genetics* **32**:393-397.
- Halpern, S. L., L. S. Adler, and M. Wink. 2010. Leaf herbivory and drought stress affect floral attractive and defensive traits in *Nicotiana quadrivalvis*. *Oecologia* **163**:961-971.
- Hambäck, P. A. 2001. Direct and indirect effects of herbivory: Feeding by spittlebugs affects pollinator visitation rates and seedset of *Rudbeckia hirta*. *Ecoscience* **8**:45-50.
- Harder, L. D. and S. C. H. Barrett. 1993. Pollen removal from tristylous *Pontederia cordata*: Effects of anther position and pollinator specialization. *Ecology* **74**:1059-1072.
- Harder, L. D. and S. C. H. Barrett. 1995. Mating cost of large floral displays in hermaphrodite plants. *Nature* **373**:512-515.
- Harder, L. D. and W. G. Wilson. 1998. A clarification of pollen discounting and its joint effects with inbreeding depression on mating system evolution. *The American Naturalist* **152**:684-695.
- Harder, L. D., N. M. Williams, C. Y. Jordan, and W. A. Nelson. 2001. The effects of floral design and display on pollinator economics and pollen dispersal. Pages 297-317 in L. Chittka and J. D. Thomson, editors. *Cognitive ecology of pollination: Animal behaviour and floral evolution*. Cambridge University Press, West Nyack, NY, USA.
- Hermann, B. P., T. K. Mal, R. J. Williams, and N. R. Dollahon. 1999. Quantitative evaluation of stigma polymorphism in a tristylous weed, *Lythrum salicaria* (Lythraceae). *American Journal of Botany* **86**:1121-1129.

- Hladun, K. R. and L. S. Adler. 2009. Influence of leaf herbivory, root herbivory, and pollination on plant performance in *Cucurbita moschata*. *Ecological Entomology* **34**:144-152.
- Hunt-Joshi, T. R., B. Blossey, and R. B. Root. 2004. Root and leaf herbivory on *Lythrum salicaria*: Implications for plant performance and communities. *Ecological Applications* **14**:1574-1589.
- JMP, version 10. SAS Institute Inc., Cary, NC.1998-2011
- Johnson, S. D. and A. Dafni. 1998. Response of bee-flies to the shape and pattern of model flowers: Implications for floral evolution in a Mediterranean herb. *Functional Ecology* **12**:289-297.
- Karban, R. and S. Y. Strauss. 1993. Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. *Ecology* **74**:39-46.
- Katovich, E. J. S., D. W. Ragsdale, L. C. Skinner, and R. L. Becker. 2001. Effect of *Galerucella* spp. feeding on seed production in purple loosestrife. *Weed Science* **49**:190-194.
- Kessler, A., R. Halitschke, and K. Poveda. 2011. Herbivory-mediated pollinator limitation: Negative impacts of induced volatiles on plant-pollinator interactions. *Ecology* **92**:1769-1780.
- King, V. M. and R. D. Sargent. 2012. Presence of an invasive plant species alters pollinator visitation to a native. *Biological Invasions* **14**:1809-1818.
- Klinkhamer, P. G. L. and T. J. de Jong. 1990. Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). *Oikos* **57**:399-405.
- Knight, T. M. 2003. Floral density, pollen limitation, and reproductive success in *Trillium grandiflorum*. *Oecologia* **137**:557-563.
- Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T. L. Ashman. 2005. Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology, Evolution, and Systematics* **36**:467-497.
- Kok, L. T., T. J. McAvoy, R. A. Malecki, S. D. Hight, J. J. Drea, and J. R. Coulson. 1992. Host specificity tests of *Galerucella californiensis* (L.) and *G. pusilla* (Duft.) (Coleoptera: Chrysomelidae), potential biological control agents of purple loosestrife, *Lythrum salicaria* L. (Lythraceae). *Biological Control* **2**:282-290.
- Krupnick, G. A. and A. E. Weis. 1998. Floral herbivore effect on the sex expression of an andromonoecious plant, *Isomeris arborea* (Capparaceae). *Plant Ecology* **134**:151-162.

- Krupnick, G. A. and A. E. Weis. 1999. The effect of floral herbivory on male and female reproductive success in *Isomeris arborea*. *Ecology* **80**:135-149.
- Krupnick, G. A., A. E. Weis, and D. R. Campbell. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology* **80**:125-134.
- Kunin, W. E. 1997. Population size and density effects in pollination: Pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *Journal of Ecology* **85**:225-234.
- Landis, D. A., D. C. Sebolt, M. J. Haas, and M. Klepinger. 2003. Establishment and impact of *Galerucella californiensis* L. (Coleoptera: Chrysomelidae) on *Lythrum salicaria* L. and associated plant communities in Michigan. *Biological Control* **28**:78-91.
- Lehtilä, K. and S. Y. Strauss. 1997. Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. *Oecologia* **111**:396-403.
- Lehtilä, K. and S. Y. Strauss. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. *Ecology* **80**:116-124.
- Levin, D. A. and H. W. Kerster. 1973. Assortative pollination for stature in *Lythrum salicaria*. *Evolution* **27**:144-152.
- Lindgren, C. J. 1999. Performance of a biological control agent, *Galerucella californiensis* L. (Coleoptera: Chrysomelidae) on purple loosestrife *Lythrum salicaria* L. in Southern Manitoba (1993-1998). Pages 367-382 in *Proceedings of the X International Symposium on Biological Control of Weeds*, Montana State University, Bozeman, Montana, USA.
- Lindgren, C. J., J. Corrigan, and R. A. De Clerck-Floate. 2002. *Lythrum salicaria* L., purple loosestrife (Lythraceae). Pages 383-390 in P. G. Mason and J. T. Huber, editors. *Biological Control Programmes in Canada, 1981-2000*. CABI Publishing, Wallingford, UK.
- Mal, T. K., J. Lovett-Doust, and L. Lovett-Doust. 1999. Maternal and paternal success among flower morphs in tristylous *Lythrum salicaria*. *Aquatic Botany* **63**:229-239.
- Mal, T. K., J. Lovett-Doust, L. Lovett-Doust, and G. A. Mulligan. 1992. The biology of Canadian weeds. 100. *Lythrum salicaria*. *Canadian Journal of Plant Science* **72**:1305-1330.
- Malecki, R. A., B. Blossey, S. D. Hight, D. Schroeder, L. T. Kok, and J. R. Coulson. 1993. Biological control of purple loosestrife. *BioScience* **43**:680-686.
- MATLAB, version 7.9.0.0529. The Mathworks Inc., Natick, MA. 1984-2009.
- Mitchell, R. J. 1994. Effects of floral traits, pollinator visitation, and plant size on *Ipomopsis aggregata* fruit production. *The American Naturalist* **143**:870-889.

- Møller, A. P. and M. Eriksson. 1995. Pollinator preference for symmetrical flowers and sexual selection in plants. *Oikos* **73**:15-22.
- Mothershead, K. and R. J. Marquis. 2000. Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. *Ecology* **81**:30-40.
- Mutikainen, P. and L. F. Delph. 1996. Effects of herbivory on male reproductive success in plants. *Oikos* **75**:353-358.
- Nicholls, M. S. 1987. Pollen flow, self-pollination and gender specialization: Factors affecting seed-set in the tristylous species *Lythrum salicaria* (Lythraceae). *Plant Systematics and Evolution* **156**:151-157.
- Niesenbaum, R. A. 1996. Linking herbivory and pollination: Defoliation and selective fruit abortion in *Lindera benzoin*. *Ecology* **77**:2324-2331.
- Nötzold, R., B. Blossey, and E. Newton. 1998. The influence of below ground herbivory and plant competition on growth and biomass allocation of purple loosestrife. *Oecologia* **113**:82-93.
- O'Neil, P. 1994. Genetic incompatibility and offspring quality in the tristylous plant *Lythrum salicaria* (Lythraceae). *American Journal of Botany* **81**:76-84.
- Ohashi, K. and T. Yahara. 1998. Effects of variation in flower number on pollinator visits in *Cirsium purpuratum* (Asteraceae). *American Journal of Botany* **85**:219-224.
- Ohashi, K. and T. Yahara. 2001. Behavioral responses of pollinators to variation in floral display size and their influences on the evolution of floral traits. Pages 274-296 in L. Chittka and J. D. Thomson, editors. *Cognitive ecology of pollination: Animal behaviour and floral evolution*. Cambridge University Press, West Nyack, NY, USA.
- Ottenbreit, K. A. and R. J. Staniforth. 1994. Crossability of naturalized and cultivated *Lythrum* taxa. *Canadian Journal of Botany* **72**:337-341.
- Pellegrino, G. and A. Musacchio. 2006. Effects of defoliation on reproductive success in two orchids, *Serapias vomeracea* and *Dactylorhiza sambucina*. *Annales Botanici Fennici* **43**:123-128.
- Poveda, K., I. Steffan-Dewenter, S. Scheu, and T. Tschardtke. 2003. Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set. *Oecologia* **135**:601-605.
- Poveda, K., I. Steffan-Dewenter, S. Scheu, and T. Tschardtke. 2005. Effects of decomposers and herbivores on plant performance and aboveground plant-insect interactions. *Oikos* **108**:503-510.

- Quesada, M., K. Bollman, and A. G. Stephenson. 1995. Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. *Ecology* **76**:437-443.
- Quinn, G. P. and M. J. Keough. 2002. *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge, UK.
- Rush, S., J. K. Conner, and P. Jennetten. 1995. The effects of natural variation in pollinator visitation on rates of pollen removal in wild radish, *Raphanus raphanistrum* (Brassicaceae). *American Journal of Botany* **82**:1522-1526.
- Schat, M. and B. Blossey. 2005. Influence of natural and simulated leaf beetle herbivory on biomass allocation and plant architecture of purple loosestrife (*Lythrum salicaria* L.). *Environmental Entomology* **34**:906-914.
- Sharaf, K. E. and M. V. Price. 2004. Does pollination limit tolerance to browsing in *Ipomopsis aggregata*? *Oecologia* **138**:396-404.
- Stanton, M. L., A. A. Snow, and S. N. Handel. 1986. Floral evolution - Attractiveness to pollinators increases male fitness. *Science* **232**:1625-1627.
- Steets, J. A. and T. L. Ashman. 2004. Herbivory alters the expression of a mixed-mating system. *American Journal of Botany* **91**:1046-1051.
- Stephenson, A. G. 1980. Fruit set, herbivory, fruit reduction, and the fruiting strategy of *Catalpa speciosa* (Bignoniaceae). *Ecology* **61**:57-64.
- Stephenson, A. G. 1981. Flower and fruit abortion: Proximate causes and ultimate functions. *Annual Review of Ecology and Systematics* **12**:253-279.
- Stout, A. B. 1923. Studies of *Lythrum salicaria* I. The efficiency of self-pollination. *American Journal of Botany* **10**:440-449.
- Strauss, S. Y. 1991. Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. *Ecology* **72**:543-558.
- Strauss, S. Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. *Ecology* **78**:1640-1645.
- Strauss, S. Y., J. K. Conner, and K. P. Lehtila. 2001. Effects of foliar herbivory by insects on the fitness of *Raphanus raphanistrum*: Damage can increase male fitness. *The American Naturalist* **158**:496-504.
- Strauss, S. Y., J. K. Conner, and S. L. Rush. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: Implications for male and female plant fitness. *The American Naturalist* **147**:1098-1107.

- Strauss, S. Y. and R. E. Irwin. 2004. Ecological and evolutionary consequences of multispecies plant-animal interactions. *Annual Review of Ecology, Evolution, and Systematics* **35**:435-466.
- Suárez, L. H., W. L. Gonzalez, and E. Gianoli. 2009. Foliar damage modifies floral attractiveness to pollinators in *Alstroemeria exerens*. *Evolutionary Ecology* **23**:545-555.
- Swope, S. M. and I. M. Parker. 2012. Complex interactions among biological control agents, pollinators, and an invasive weed: A structural equation modeling approach. *Ecological Applications* **22**:2122-2134.
- Thompson, D. W., R. L. Stuckey, and E. B. Thompson. 1987. Spread, impact and control of purple loosestrife (*Lythrum salicaria*) in North American wetlands. Fish and Wildlife Research 2. U.S. Department of the Interior, Fish and Wildlife Service, Washington D.C.
- Thomson, V. P., A. B. Nicotra, and S. A. Cunningham. 2004. Herbivory differentially affects male and female reproductive traits of *Cucumis sativus*. *Plant Biology* **6**:621-628.
- Van Zandt, P. A. and A. A. Agrawal. 2004. Specificity of induced plant responses to specialist herbivores of the common milkweed *Asclepias syriaca*. *Oikos* **104**:401-409.
- Vázquez, D. P. and D. Simberloff. 2004. Indirect effects of an introduced ungulate on pollination and plant reproduction. *Ecological Monographs* **74**:281-308.
- Venez, J. I. and L. W. Aarssen. 1998. Effects of shoot apex removal in *Lythrum salicaria* (Lythraceae): Assessing the costs of reproduction and apical dominance. *Annales Botanici Fennici* **35**:101-111.
- Voelckel, C. and I. T. Baldwin. 2004. Generalist and specialist lepidopteran larvae elicit different transcriptional responses in *Nicotiana attenuata*, which correlate with FAC profiles. *Ecology Letters* **7**:770-775.
- Willson, M. F. and N. Burley. 1983. Mate choice in plants: Tactics, mechanisms, and consequences. Princeton University Press, New Jersey, USA.
- Wolf, L. L. and F. R. Hainsworth. 1990. Non-random foraging by hummingbirds: Patterns of movement between *Ipomopsis aggregata* (Pursh) V. Grant inflorescences. *Functional Ecology* **4**:149-157.
- Wolfe, L. M. and S. C. H. Barrett. 1989. Patterns of pollen removal and deposition in tristylous *Pontederia cordata* L. (Pontederiaceae). *Biological Journal of the Linnean Society* **36**:317-329.

- Yakimowski, S. B., H. A. Hager, and C. G. Eckert. 2005. Limits and effects of invasion by the nonindigenous wetland plant *Lythrum salicaria* (purple loosestrife): a seed bank analysis. *Biological Invasions* 7:687-698.
- Zamora, R., J. A. Hódar, and J. M. Gómez. 1999. Plant-herbivore interaction: Beyond a binary vision. Pages 677-718 in F. Pugnaire and F. Valladares, editors. *Functional plant ecology*. Marcel Dekker Inc., New York.

Table 2.1. Least-squares regressions among plant traits and aspects of pollinator visitation.

Note: some results are not presented because residuals were not normally distributed.

Aspect of pollinator visitation	Plant trait			
		No. flowers	No. inflorescences	Height (cm)
Mean No. foraging bouts per 30 min ³	R^2 adj	0.57	0.46	0.31
	$F_{(1,81)}$	111.50	71.83	36.12 ¹
	p	< 0.0001	< 0.0001	< 0.0001
Mean No. flowers probed per 30 min ³	R^2 adj	0.74	0.60	0.30
	$F_{(1,81)}$	229.44	121.84	35.11 ¹
	p	< 0.0001	< 0.0001	< 0.0001
Mean No. flowers probed per foraging bout ⁴	R^2 adj	0.40	0.29	0.06
	$F_{(1,72)}$	50.15	30.17	6.00
	p	< 0.0001	< 0.0001	0.0167
Mean No. switches among inflorescences per foraging bout ³	R^2 adj	0.49		
	$F_{(1,72)}$	72.51		
	p	< 0.0001		

¹ $F_{(1,79)}$, not $F_{(1,81)}$

² $F_{(1,71)}$, not $F_{(1,72)}$

³Response variable was square root transformed.

⁴Response variable was log transformed.

Table 2.2. Results of an ANCOVA of the effects of herbivory treatment and flowering plant community on the number of foraging bouts performed by pollinators to individual *L. salicaria* plants during 30-minute periods. Plants were the unit of replication and the ANCOVA was weighted by the number of observation periods per plant. Note: the response variable was square root transformed.

Source	<i>Df</i>	<i>SS</i>	<i>F</i>	<i>P</i>
Treatment	2	7.5794	1.8161	0.1696
No. non-focal flowering stems ¹	1	42.5116	20.3720	<0.0001
Treatment * No. non-focal inflorescences ¹	2	8.2405	1.9745	0.1458

¹The number of non-focal *L. salicaria* and *D. verticillatus* flowering stems within a 0.38 m² plot around the focal plant.

Table 2.3. Results of an ANCOVA of the effects of herbivory treatment and flowering plant community on the number of *L. salicaria* flowers probed by pollinators during 30-minute periods. Plants were the unit of replication and the ANCOVA was weighted by the number of observation periods per plant. Note: the response variable was square root transformed.

Source	<i>Df</i>	<i>SS</i>	<i>F</i>	<i>P</i>
Treatment	2	100.1785	2.9344	0.0591
No. non-focal flowering stems ¹	1	112.4217	6.5860	0.0122
Treatment * No. non-focal inflorescences ¹	2	103.9282	3.0422	0.0534

¹ The number of non-focal *L. salicaria* and *D. verticillatus* flowering stems within a 0.38 m² plot around the focal plant.

Table 2.4. Results of an ANCOVA of the effects of herbivory treatment and flowering plant community on the number of *L. salicaria* flowers probed by pollinators during individual foraging bouts. Plants were the unit of replication and the ANCOVA was weighted by the number of observation periods per plant. Note: the response variable was log transformed.

Source	<i>Df</i>	<i>SS</i>	<i>F</i>	<i>P</i>
Treatment	2	4.5694	3.4242	0.0383
No. non-focal flowering stems ¹	1	0.2740	0.4107	0.5238
Treatment * No. non-focal inflorescences ¹	2	2.9080	2.1792	0.1210

¹ The number of non-focal *L. salicaria* and *D. verticillatus* flowering stems within a 0.38 m² plot around the focal plant.

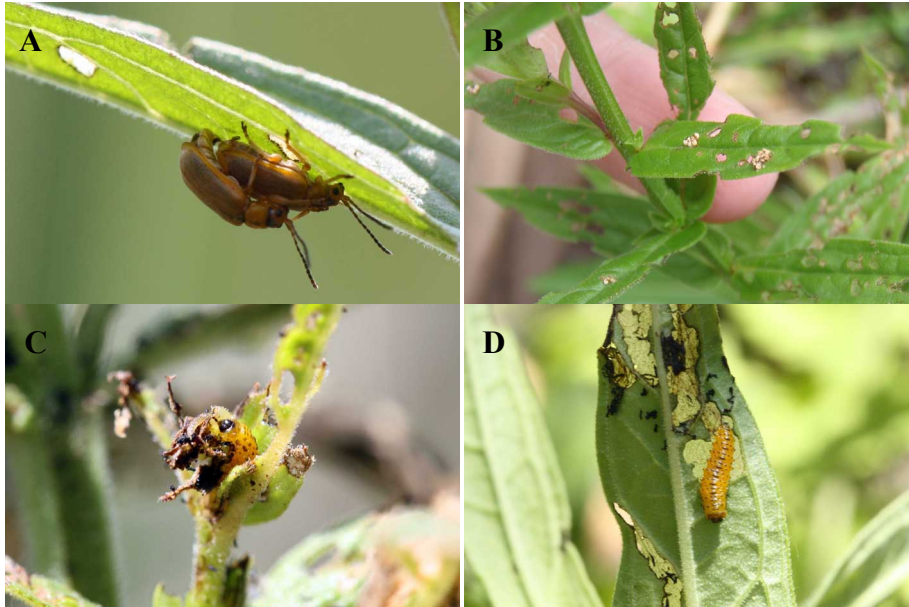


Figure 2.1. Photographs of various life-history stages of *Galerucella* beetles observed at Cow Island Marsh in 2011: A) adults mating, B) eggs oviposited on *L. salicaria* leaves (courtesy of K. Fowler), C) larva feeding on *L. salicaria* meristem, and D) larva feeding on *L. salicaria* leaf.



Figure 2.2. Photographs of A) a section of the study site, Cow Island Marsh, with multiple *L. salicaria* stems covered with no-see-um netting and B) a close-up of an individual *L. salicaria* stem covered with no-see-um netting.

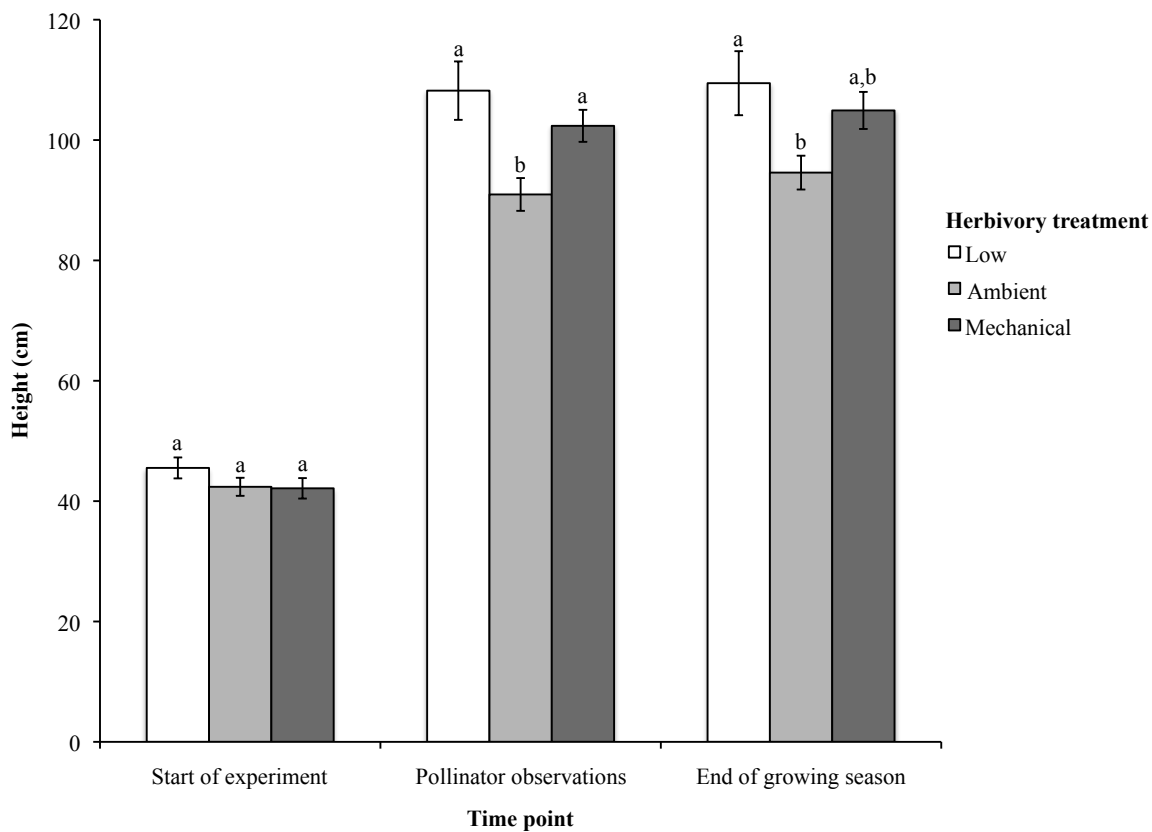


Figure 2.3. Mean (\pm SE) height of *L. salicaria* plants among three herbivory treatments at three points in time. Note: lettering is specific to each time point and does not make comparisons among time points. Bars that are not connected by the same letter are significantly different ($p < 0.05$).

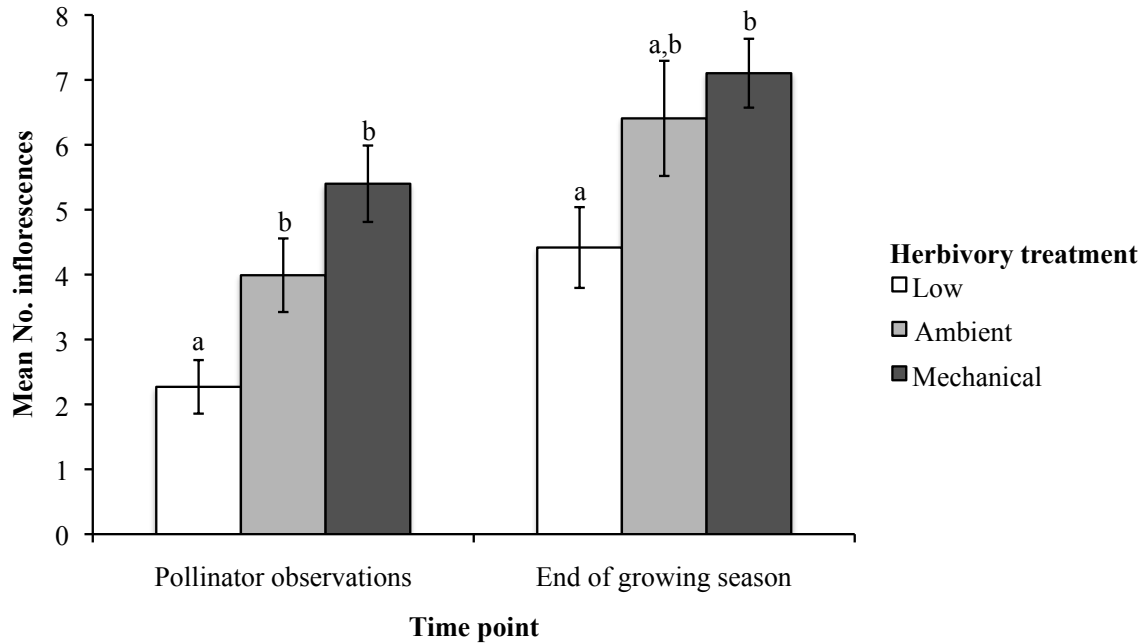


Figure 2.4. Mean (\pm SE) number of inflorescences on *L. salicaria* plants in three herbivory treatments during pollinator observations and by the end of the growing season. The total number of inflorescences produced by the end of the growing season was inferred from the total number of fruiting stalks 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 ($p < 0.05$).

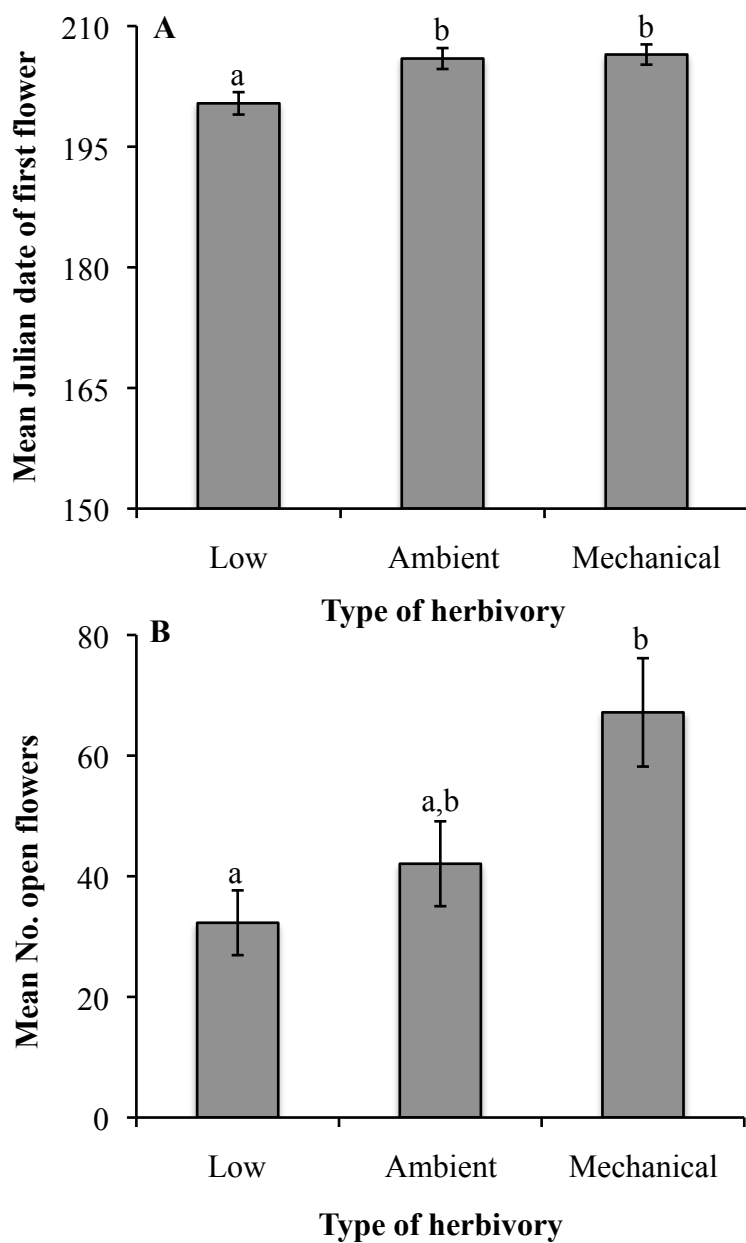


Figure 2.5. Mean (\pm SE) (A) Julian date of first flower ($n = 76$) and (B) number of open flowers during pollinator observations ($n = 83$) in *L. salicaria* plants in three herbivory treatments. Note: lettering is specific to each panel. Bars that are not connected by the same letter are significantly different ($p < 0.05$).

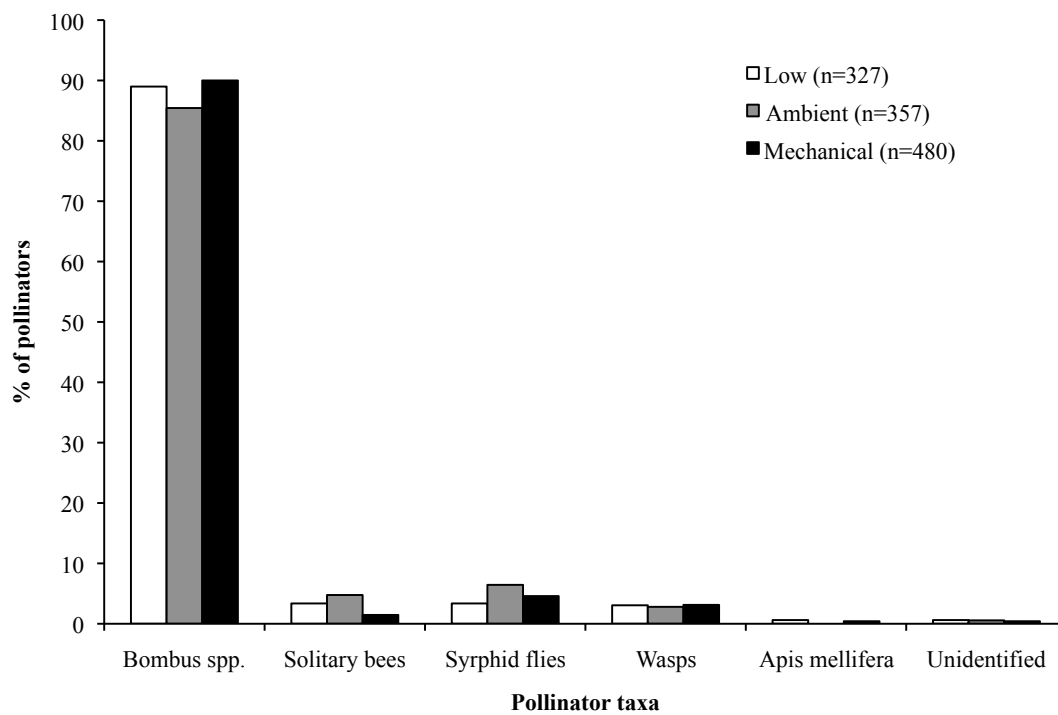


Figure 2.6. Taxonomic composition of pollinators that visited *L. salicaria* plants in three herbivory treatments (n = total number of pollinators observed within each herbivory treatment).

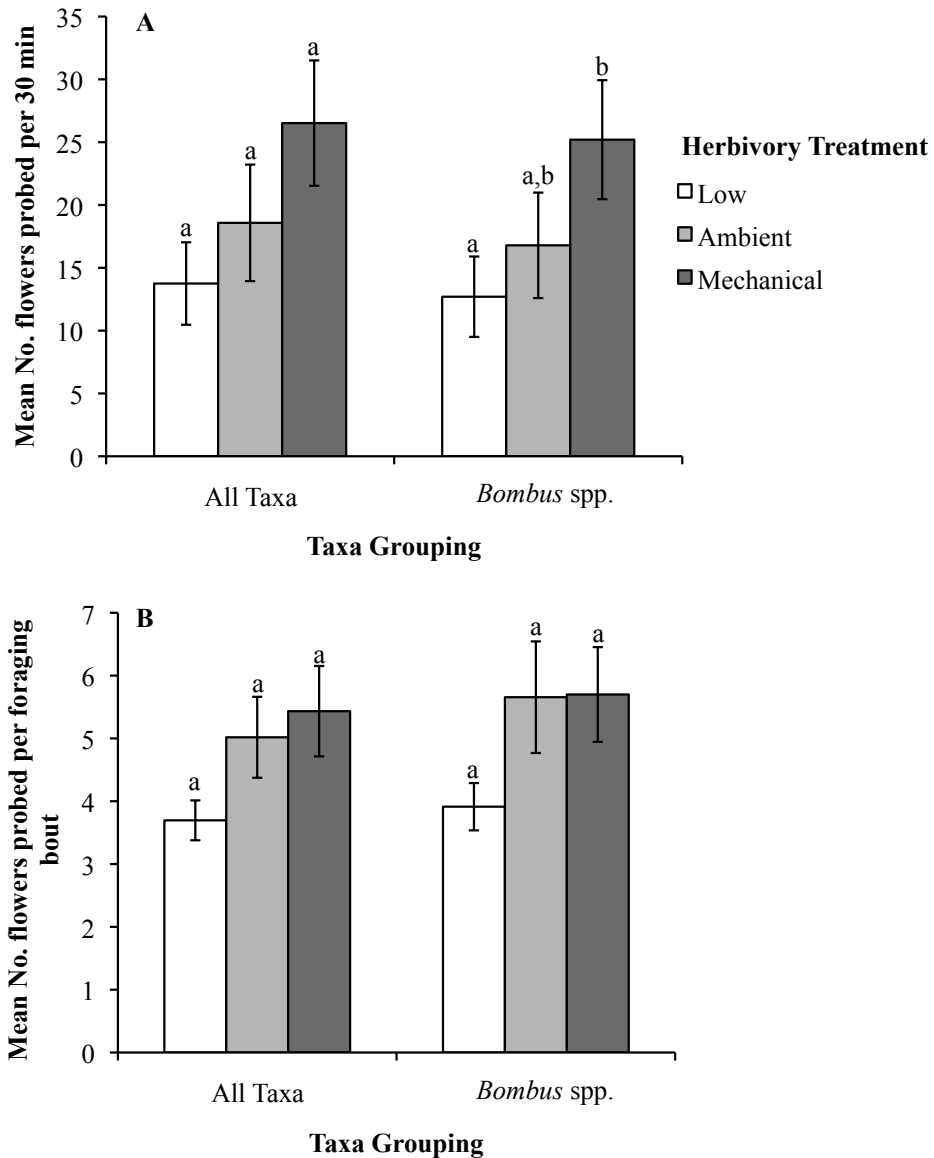


Figure 2.7. Mean (\pm SE) number of flowers probed, grouped by all pollinator taxa and by *Bombus* spp., (A) per 30 minutes and (B) per foraging bout on *L. salicaria* plants in three herbivory treatments. Note: lettering is specific to each taxa grouping within each panel and does not make comparisons among the taxa groupings. Bars that are not connected by the same letter are significantly different ($p < 0.05$).

CHAPTER 3: Conclusions and Future Directions

The work presented here demonstrates that *Galerucella* beetle herbivory can result in changes to floral traits that are correlated with changes in pollinator visitation to *L. salicaria*. Differences in pollinator visitation were likely mediated by the direct effects of herbivory on the size and architecture of floral displays. While herbivory apparently indirectly altered pollination, there was no detectable effect on fruit or seed production. Future research should focus on additional mechanisms by which *Galerucella* beetles may affect fitness in *L. salicaria*. The areas of research described below will help to elucidate the effects of *Galerucella* on total plant fitness, will increase our ability to predict the effectiveness of this species as a biological control agent, and will add to the growing body of literature on multispecies interactions.

A further understanding of the effects of herbivory on *L. salicaria* reproductive success may be gained by examining offspring quality. If increases in the number of flowers probed per pollinator caused higher rates of within plant fertilization, then I would expect to see differences in seedling quality (height, number and size of leaves (see O'Neil 1994)).

Pollen limitation, or a lack thereof, may have contributed to the observation of no effect of herbivory on fruit or seed production. If there is no impact of herbivory on pollen limitation, this limits the potential for related changes in pollinator-mediated interactions to influence female fitness. Thus, future research should pair an experiment similar to the one described in this thesis with a pollen supplementation experiment (Burd 1994, Ashman 2004), where some plants receive hand-applied outcross pollen and other plants are open pollinated (naturally by the pollinator community) (e.g., Mothershead and Marquis 2000, Kessler et al. 2011). Such an experiment will determine if reproduction is limited by

pollinator visitation and if there are differences in the degree of pollen limitation between plants exposed and plants unexposed to herbivory (Burd 1994).

It is not known if the observed responses in our study population to *Galerucella* feeding could vary under different environments (e.g., Halpern et al. 2010, Brody and Irwin 2012). For example, ovule, and therefore seed, production may be limited by resource levels or patterns of allocation (Stephenson 1981, Willson and Burley 1983, Niesenbaum 1996), which can be negatively impacted by herbivory (Lehtilä and Strauss 1999). Furthermore, Halpern et al. (2010) noted that their recent work suggests that plant responses to herbivory may be constrained by the environment. For example, in their study, a response of *Nicotiana quadrivalis* plants to herbivory (increased nectar production) was not expressed in a treatment that mimicked drought conditions, but was expressed in a treatment with high levels of watering. Future studies should focus on the potential for herbivory by abiotic interactions through a fully-factorial experiment crossing herbivory with manipulations of physical conditions, such as water levels and nutrient availability, as these factors can influence plant growth (e.g., Brody and Irwin 2012). Such an experiment would determine whether responses observed in the present study vary with environmental conditions.

Examining effects of herbivory on male fitness, i.e., pollen export, which was not examined, but which is expected to be more sensitive to differences in pollinator visitation than female fitness (Bateman 1948, Willson and Burley 1983, Bell 1985, Stanton et al. 1986), would make an excellent next step. As previously discussed, herbivory may lead to a reduction in pollen production (Frazee and Marquis 1994, Quesada et al. 1995, Strauss et al. 1996, Lehtilä and Strauss 1999), which will decrease the maximum potential number of seeds that can be sired by a given male (Delph et al. 1997) and may also affect pollinator visitation, which in itself may affect the number of seeds sired (Strauss 1997). Moreover,

reduced allocation may decrease the competitive ability of pollen, relative to plants that are not fed upon by herbivores (Quesada et al. 1995, Mutikainen and Delph 1996). Also, in the current study, an increase in the number of flowers probed per unit time in the high and ambient herbivory treatments, compared to the low herbivory treatment, may have positively impacted male fitness (e.g., increased total outcrossing (Harder et al. 2001)). However, because each pollinator visited more flowers per foraging bout, male fitness may have been negatively impacted in the ambient and mechanical herbivory treatments relative to the low herbivory treatment due to increased pollen discounting, as described in Chapter 1 (Strauss 1997, Harder and Wilson 1998, Ohashi and Yahara 2001, Harder et al. 2001, Biernaskie and Cartar 2004).

Future studies should also focus on identifying the specific floral traits that are key to pollinator visitation in this system. To achieve this, experiments could be designed to manipulate specific floral traits while controlling for others. For example, manipulating the number of open flowers while keeping the number of inflorescences constant would serve as a test of the hypothesis that differences in the number of flowers probed among treatments were largely mediated by differences in the number of open flowers. Also, it would be important to determine if pollinators are responding to additional cues that may be affected by herbivory, such as nectar volume (Krupnick et al. 1999, Halpern et al. 2010).

Assortative mating by height can occur in *L. salicaria*; one study found that bees tend to switch among inflorescences that vary by less than approximately 6.35 cm in height (Levin and Kerster 1973). Therefore, it would be useful to determine whether the changes to plant height resulting from herbivory also influence gene flow among individuals that suffer different levels of herbivore damage. Plants in the low herbivory treatment of the present study averaged 17.25 cm and 5.85 cm taller than plants in the ambient and mechanical

treatments, respectively, which opens the potential for assortative mating for damage history to occur. However, it is important to note that alterations in gene flow among my treatments, due to differences in height, may be weak or non-existent for several reasons. First, plant height in my study was based on the tallest inflorescence on each plant, but plants generally produce multiple inflorescences. Thus, although there was a significant effect of treatment on the height of the tallest inflorescence, there may be a greater extent of overlap when the distributions of the heights of other inflorescences are considered. Second, the variation within each treatment was such that there was some degree of overlap in maximum plant height among treatments.

Plant responses to herbivory vary depending on the intensity of feeding damage (Doak 1992). For example, Schat and Blossey (2005) explored the impact of the percentage of *L. salicaria* leaf removed by herbivores (by manipulating *Galerucella* larval density) and found that there was a significant negative relationship between plant height and the percent of leaf area removed. In the same study, as the level of damage increased, the total length of inflorescences decreased, while the total number of inflorescences increased. Thus, traits that are important for altering pollinator behavior (e.g., total inflorescence length and hence the number of flowers) can vary with the extent of herbivore induced damage. It is therefore reasonable to expect that the indirect effects of herbivory (altered pollinator visitation) may also be influenced by the degree of herbivory. It would be useful to experimentally alter the extent of herbivory damage to determine the threshold level of herbivory, above which various aspects of *L. salicaria* fitness are negatively affected.

The influence of the local ecological community on the response of *L. salicaria* to *Galerucella* herbivory is an additional direction of future research. For example, neighbouring plants may compete with *L. salicaria*, possibly altering the direct responses of

L. salicaria to herbivory (e.g., Hjalten et al. 1973, Boege 2010). Furthermore, the plant community may affect the rate of pollinator visitation to plants that are damaged by herbivores. Indeed, I found that there was significant positive linear relationship between the density of co-flowering species and the number of foraging bouts and the number of flowers probed per 30-minute period. Thus, in cases where herbivory reduces pollinator visitation (e.g., decreased flower production, degraded flower appearance, etc.), the surrounding plant community may ameliorate the reduced attractiveness of plants and influence the number of pollinators that visit a patch of plants. Furthermore, it would be useful to determine if plant community composition alters the efficacy of *Galerucella* beetles as a biological control agent. For example, Hambäck et al. (2000) found that the presence of the plant *Myrica gale*, which may make *L. salicaria* more difficult to locate, reduces the extent of *Galerucella* damage to *L. salicaria*.

The empirical work presented in this thesis demonstrates that feeding by *Galerucella* beetles, an introduced biological control agent, can affect pollinator visitation to the invasive *L. salicaria*. This effect is likely mediated by changes in plant architecture and floral display size. Increased pollinator visitation to plants in the ambient and mechanical herbivory treatments did not lead to increased fruit or seed production. However, it remains unclear whether an increase in the number of visits per unit time and per foraging bout had the effect of increasing or decreasing overall plant fitness. Future work on the areas highlighted in this chapter will increase our understanding of the effects of herbivory on pollinator-mediated plant fitness in this system. In particular, examining the indirect effects of herbivory on male and female fitness, mediated by pollinators, will reveal whether the negative impacts of *Galerucella* feeding on *L. salicaria* (e.g., reduced height, delayed onset of flowering, etc.) are exacerbated (i.e., increased selfing) or partly ameliorated (i.e., increased outcrossed siring

success) by differences in pollinator visitation following herbivory (see Morris et al. 2007). Furthermore, these areas of research should elucidate the conditions (e.g., extent of feeding damage; water and nutrient availability; and community composition) under which *L. salicaria*'s responses to herbivory, and thus plant-pollinator interactions, may differ.

References

- Ashman, T. L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, S. J. Mazer, R. J. Mitchell, M. T. Morgan, and W. G. Wilson. 2004. Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology* **85**:2408-2421.
- Barber, N. A., L. S. Adler, and H. L. Bernardo. 2011. Effects of above- and belowground herbivory on growth, pollination, and reproduction in cucumber. *Oecologia* **165**:377-386.
- Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* **2**:349-368.
- Bell, G. 1985. On the function of flowers. *Proceedings of the Royal Society of London B: Biological Sciences* **224**:223-265.
- Boege, K. 2010. Induced responses to competition and herbivory: Natural selection on multi-trait phenotypic plasticity. *Ecology* **91**:2628-2637.
- Brody, A. K. and R. E. Irwin. 2012. When resources don't rescue: Flowering phenology and species interactions affect compensation to herbivory in *Ipomopsis aggregata*. *Oikos* **121**:1424-1434.
- Delph, L. F., M. H. Johannsson, and A. G. Stephenson. 1997. How environmental factors affect pollen performance: Ecological and evolutionary perspectives. *Ecology* **78**:1632-1639.
- Doak, D. F. 1992. Lifetime impacts of herbivory for a perennial plant. *Ecology* **73**:2086-2099.
- Frazer, J. E. and R. J. Marquis. 1994. Environmental contribution to floral trait variation in *Chamaecrista fasciculata* (Fabaceae, Caesalpinioideae). *American Journal of Botany* **81**:206-215.
- Halpern, S. L., L. S. Adler, and M. Wink. 2010. Leaf herbivory and drought stress affect floral attractive and defensive traits in *Nicotiana quadrivalvis*. *Oecologia* **163**:961-971.
- Hambäck, P. A., J. Ågren, and L. Ericson. 2000. Associational resistance: Insect damage to purple loosestrife reduced in thickets of sweet gale. *Ecology* **81**:1784-1794.
- Harder, L. D., N. M. Williams, C. Y. Jordan, and W. A. Nelson. 2001. The effects of floral design and display on pollinator economics and pollen dispersal. Pages 297-317 in L. Chittka and J. D. Thomson, editors. *Cognitive ecology of pollination: Animal behaviour and floral evolution*. Cambridge University Press, West Nyack, NY, USA.

- Hjälten, J., K. Danell, and L. Ericson. 1993. Effects of simulated herbivory and intraspecific competition on the compensatory ability of birches. *Ecology* **74**:1136-1142.
- Kessler, A., R. Halitschke, and K. Poveda. 2011. Herbivory-mediated pollinator limitation: Negative impacts of induced volatiles on plant-pollinator interactions. *Ecology* **92**:1769-1780.
- Krupnick, G. A., A. E. Weis, and D. R. Campbell. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology* **80**:125-134.
- Lehtilä, K. and S. Y. Strauss. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. *Ecology* **80**:116-124.
- Levin, D. A. and H. W. Kerster. 1973. Assortative pollination for stature in *Lythrum salicaria*. *Evolution* **27**:144-152.
- Morris, W. F., R. A. Hufbauer, A. A. Agrawal, J. D. Bever, V. A. Borowicz, G. S. Gilbert, J. L. Maron, C. E. Mitchell, I. M. Parker, G. P. Alison, M. E. Torchin, and D. P. Vázquez. 2007. Direct and interactive effects of enemies and mutualists on plant performance: A meta-analysis. *Ecology* **88**:1021-1029.
- Mothershead, K. and R. J. Marquis. 2000. Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. *Ecology* **81**:30-40.
- Mutikainen, P. and L. F. Delph. 1996. Effects of herbivory on male reproductive success in plants. *Oikos* **75**:353-358.
- Niesenbaum, R. A. 1996. Linking herbivory and pollination: Defoliation and selective fruit abortion in *Lindera benzoin*. *Ecology* **77**:2324-2331.
- O'Neil, P. 1994. Genetic incompatibility and offspring quality in the tristylous plant *Lythrum salicaria* (Lythraceae). *American Journal of Botany* **81**:76-84.
- Poveda, K., I. Steffan-Dewenter, S. Scheu, and T. Tschardt. 2003. Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set. *Oecologia* **135**:601-605.
- Quesada, M., K. Bollman, and A. G. Stephenson. 1995. Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. *Ecology* **76**:437-443.
- Stanton, M. L., A. A. Snow, and S. N. Handel. 1986. Floral evolution: Attractiveness to pollinators increases male fitness. *Science* **232**:1625-1627.
- Stephenson, A. G. 1981. Flower and fruit abortion: Proximate causes and ultimate functions. *Annual Review of Ecology and Systematics* **12**:253-279.

- Strauss, S. Y., J. K. Conner, and S. L. Rush. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: Implications for male and female plant fitness. *The American Naturalist* **147**:1098-1107.
- Strauss, S. Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. *Ecology* **78**:1640-1645.
- Willson, M. F. and N. Burley. 1983. *Mate choice in plants: Tactics, mechanisms, and consequences*. Princeton University Press, New Jersey, USA.