

Dissertation

INDIRECT EFFECTS IN PLANT-POLLINATOR INTERACTIONS: THE ROLE OF  
EXOTIC PLANTS AND HERBIVORES

Submitted by

Daniel Paul Cariveau

Graduate Degree Program in Ecology

In partial fulfillment of the requirements for the

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Colorado State University

Fort Collins, CO 80523

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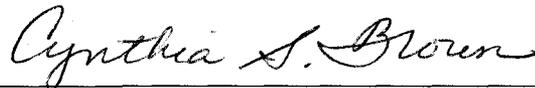
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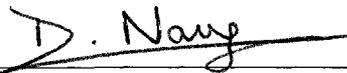
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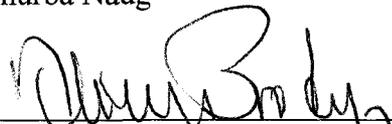
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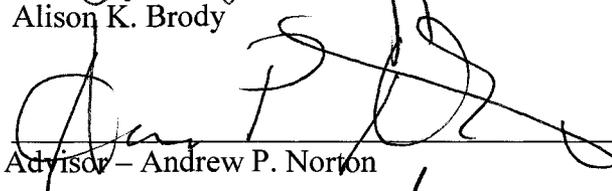
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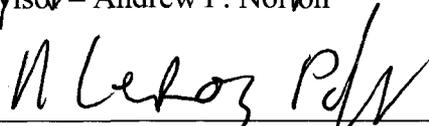
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Alison K. Brody



Advisor - Andrew P. Norton



Department Head - N. LeRoy Poff

## ABSTRACT OF DISSERTATION

### INDIRECT EFFECTS IN PLANT-POLLINATOR INTERACTIONS: THE ROLE OF EXOTIC PLANTS AND HERBIVORES

Flowering plants interact with a variety of other species. While numerous studies have demonstrated that pair-wise interactions between species are important factors affecting plant ecology and evolution, interaction with one species may affect the outcome of the interaction with another. I examined how pollination is influenced by both competition and herbivory. In Chapter One, I tested whether the presence of an exotic plant, musk thistle (*Carduus nutans* L. (Asteraceae)) influenced flower visitor behavior in relation to the native plant, bee balm (*Monarda fistulosa* L. (Lamiaceae)). I found that visitation rate to the native was not affected by the presence of the exotic. However, flower visitors commonly switched between the native and exotic and transferred exotic pollen to native plant stigmas. Conspecific pollen on the native plant stigmas was also reduced in the presence of the exotic. Seed set of the native plant was not affected. In a separate experiment, I examined how distance from the exotic plant influenced visitation rate to the native plant. I found that visitation rate to the native plant was reduced when the native plant was 1 and 5 meters from the exotic. However, visitation rate remained unchanged at 0 and 15 meters. This suggests that magnitude of interactions between plants through flower visitors may depend on spatial scale. In Chapter Two, I examined how the exotic plant, musk thistle, influenced visitation rate to the native plant, common harebell (*Campanula rotundifolia* L. (Campanulaceae)). I found that visitation rate to the native plant was reduced in the presence of the exotic plant. However, only solitary bees

exhibited a reduction in visitation rate while *Bombus* species did not. Flower visitors did not switch between the exotic and native plants, and there were no exotic pollen grains on the native plant stigmas. Conspecific pollen deposition and seed set were not affected by the presence of the exotic plant. In Chapter Three, I explored whether the biological control *Mecinus janthinus* (Coleoptera) affected floral display size and visitation rate to the exotic plant Dalmatian toadflax (*Linaria dalmatica* (Scrophulariaceae)). In addition, I examined whether *M. janthinus* feeding affected female reproductive success directly or indirectly through flower visitors. I found that herbivory decreased the number of flowers and visitation rate to Dalmatian toadflax. However, I found no effect of herbivory on seed set when conducting hand-pollinations, suggesting no indirect effects of *M. janthinus* through flower visitors.

Daniel Paul Cariveau  
Graduate Degree Program in Ecology  
Colorado State University  
Fort Collins, CO 80523  
Fall 2008

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CHAPTER ONE

SPATIALLY CONTINGENT INTERACTIONS BETWEEN AN EXOTIC AND  
NATIVE PLANT MEDIATED THROUGH FLOWER VISITORS

## Abstract

Exotic plants can negatively impact the fitness of native plants by changing the behavior of flower visitors and thus affecting pollen transfer. The presence of an exotic plant may decrease the visitation rate to native plants and thus increase pollen limitation. Flower visitors may also switch between exotic and native plants and if pollen from an exotic plant is transferred to native plant stigmas this may impede siring by conspecific pollen. As flower visitors forage within a spatial context, the distribution of plants may affect the type and magnitude of pollinator-mediated competition. In this study I examined two questions: 1) Does the exotic plant, *Carduus nutans* (Asteraceae) interact with the native *Monarda fistulosa* (Lamiaceae) through flower visitors by changing visitation rate and/or through heterospecific pollen transfer, and does this affect seed set of the native plant? 2) Does spatial context affect how the native and exotic plants interact through flower visitors? I created plots containing potted *M. fistulosa* with and without the presence of potted *C. nutans*. In the presence of *C. nutans*, *M. fistulosa* stigmas had significantly fewer conspecific and more *C. nutans* pollen grains. Visitation rate and seed set tended to be lower in these invaded plots, however they were not significant. In a second experiment, I examined whether changes in visitation rate to *M. fistulosa* due to the presence of *C. nutans* was a function of *M. fistulosa* distance from *C. nutans*. I found that visitation rate did not decrease in the presence of *C. nutans* when *M. fistulosa* were adjacent to *C. nutans* or 15 meters from *C. nutans*. However, floral visitation rate to *M. fistulosa* decreased at 1 and 5 meters from *C. nutans*. These results suggest interactions between plant species through flower visitors may depend on spatial scale.

## **Introduction**

Many plant species interact through shared mutualists and this can lead to fitness and population-level consequences (for reviews see Mitchell et al. 2006, Traveset and Richardson 2006, Bjerknes et al. 2007). For example, one plant species may reduce seed set of second plant species by affecting flower visitor behavior and decreasing quantity and quality of pollen received (e.g. Brown et al. 2002). This competition mediated through flower visitors can occur through two mechanisms. One of these mechanisms, competition for flower visitor preference, results when visitation rates and thus the amount of pollen received is reduced due to greater attraction to heterospecific plants by flower visitors. As many flowering plants do not receive enough pollen to maximize seed production (Burd 1994, Larson and Barrett 2000, Ashman et al. 2004), fitness may be reduced due to the presence of a heterospecific plant. Plants may also experience competition through heterospecific pollen transfer if flower visitors switch among plant species and deposit heterospecific pollen grains that impede the placement or germination of conspecific pollen or result in premature stigmatic closure (Waser and Fugate 1986). In addition, when flower visitors switch to a second species during a single feeding bout, the pollen from the first species may be lost to the second species. If such switching is ubiquitous, this may decrease the total availability of pollen of the first species and thus increase pollen limitation of seed set (Campbell and Motten 1985). Conversely, plants may experience an increase in fitness if the presence of one or more plant species leads to an increase in flower visitors and higher visitation rates and thus more pollen deposited than a plant would receive if alone (Rathcke 1988, Laverly 1992, Ghazoul 2006).

Floral displays of many exotic plants are large and conspicuous and offer high rewards to flower visitors (Chittka and Schurkens 2001, Ghazoul 2002, Totland et al. 2006, Bjerknes et al 2007). Also native flower visitors are often generalists and readily visit exotic plants (Memmott and Waser 2002). Therefore large floral displays of many exotic plants and the prevalence of generalist pollinators suggest that competition mediated through flower visitors may be common between exotic and native plants. However, studies of flower visitor-mediated competition between native and exotic plants have found a variety of effects including competitive interactions (Grabas and Lavery 1999, Chittka and Schurkens 2001, Brown et al. 2002, Bartomeus et al. 2008) while others found no impact (Ghazoul 2004, Jones 2004, Moragues and Traveset 2005, Totland et al. 2006) or even facilitative effects (Grabas and Lavery 1999, Moragues and Traveset 2005, Lopezaraiza-Mikel et al. 2007, Bartomeus et al. 2008; see Bjerknes et al. 2007 for review).

Competition mediated through flower visitors may occur over larger spatial scales than competition for other resources. Most traditional hypotheses regarding competition among native and exotic plants consider only competition among plants that occur in close enough proximity to interact through root by root interactions or through shading (Levine et al. 2003). Flower visitors forage at larger spatial scales, and the importance of interactions among plants through flower visitors may change with spatial context. For example, flower visitors may show preference for one species over another only when the two species are in close proximity. In this case, differences in visitation rate would increase as the distance between plants decreased. Conversely, the presence of a second plant species may attract more flower visitors overall and lead to an increase in visitation

rate as distance decreases. However, flower visitors may view plants in close proximity as a single patch of resources and not distinguish among plants with similar flower phenotypes despite differences in the amount of available rewards (Klinkhamer et al. 2001). Thus, in close proximity, interspecific floral switching may occur frequently, and this could result in competition through heterospecific pollen transfer despite an overall increase in flower visitors. These three processes are likely to be spatially dependant, and the relative importance of competition for visitor preference, heterospecific pollen transfer and overall flower visitor abundance will change with the distance among flower patches. I know of no studies that have examined how spatial proximity of native and exotic plants may influence interactions through flower visitors.

In this study, I used experimental plots to test whether the exotic musk thistle (*Carduus nutans* L. Asteraceae) interacts with the native plant bee balm (*Monarda fistulosa* L. Lamiaceae) through flower visitors. Specifically, I addressed two questions: 1) Does the exotic plant, *Carduus nutans* interact with the native *Monarda fistulosa* through flower visitors through changes in visitation rate and/or heterospecific pollen transfer and does this affect seed set of the native plant? 2) How does spatial context affect the interactions between native and exotic plants through flower visitors?

## **Methods**

### Study Species

*Carduus nutans* is native to southern Europe and Asia and is now invasive throughout much of North America, New Zealand, Australia, and South America. Throughout Colorado, it is a biennial and reproduces solely through seed production. Seeds germinate in the late summer or early fall and plants overwinter as rosettes before

blooming the following year. This thistle reaches heights of up to 2 m and produces a large floral display consisting of 3 to 10 simultaneously blooming actinomorphic inflorescences, each approximately 5 cm in diameter.

*Monarda fistulosa* is a common perennial forb present throughout much of northern North America. Individual *M. fistulosa* flowers are zygomorphic and 15 to 35 flowers occur on each inflorescence. *Monarda fistulosa* inflorescences are similar in size and shape to *C. nutans*. As with *C. nutans*, flower visitors are able to remain on the tops of the *M. fistulosa* inflorescences and probe inside the relatively short corollas. *Carduus nutans* and *M. fistulosa* receive visits from many of the same insect species (Cariveau unpublished data). *Monarda fistulosa* is self-compatible, yet seed set is enhanced by flower visitors (Cruden et al. 1984). Both plant species bloom from early July until mid-September and co-occur in close proximity in many locations in Colorado. Populations of both plants vary in abundance. Small conspecific patches of either species are found yet both most commonly occur in large patches (Cariveau pers. obs.). In particular, *C. nutans* can quickly invade disturbed areas and reach large, dense monospecific stands. This plant is listed as a noxious weed in the state of Colorado.

#### Plant Material

I collected *Carduus nutans* rosettes in the early spring of 2005 and grew them in 10 L pots on the Colorado State University campus. I purchased *M. fistulosa* seedlings in 2004 from a native plant nursery in Colorado and transplanted them into 8 L pots outside. *Monarda fistulosa* did not flower until the spring of 2005. A few plants produced flowers before the start of the experiment; however, I removed all open and thus potentially pollinated flowers prior to the experimental manipulations.

## Small-plot Experiments

This experiment took place in 2005 in the Arapahoe-Roosevelt National Forest in Larimer County, Colorado, USA at an elevation of approximately 2500 m. The study site consisted of open grass meadows interspersed among *Pinus ponderosa*, *Pseudotsuga menziesii*, and *Pinus contorta*. Within these meadows, I constructed 20 2.5 x 2 m plots each containing an array of potted plants. I used potted plants to eliminate rhizosphere interactions among plants. Each plot was separated from its nearest neighboring plot by at least 100 m and the nearest natural population of *C. nutans* was located more than 4 km from the study site. The nearest known populations of *Monarda fistulosa* were more than 1 km from the study site.

I randomly assigned 10 plots as control plots and 10 as treatment plots. Control plots contained three potted *M. fistulosa* while treatment plots contained three potted *M. fistulosa* and three potted *C. nutans*. The plot sizes included in this study represent the lower range of population sizes for each species. Within plots, each plant was separated by 1 m. I chose to maintain a constant number of native plants in treatment and control plots to isolate the effect of *C. nutans* from the effect of *M. fistulosa* plant number. A number of studies have demonstrated that an increase in the number of conspecific flowers in a patch may increase visitation and fertility (e.g. Moeller and Geber 2005). This design is thus similar to most other research on invasive and native plant interactions through flower visitors as most of these studies either add or remove invasive plants and thus keep native plant density constant (i.e. Chittka and Shurkens 2001, Totland et al. 2006, Lopezaraiza-Mikel et al. 2007). I prevented spread of *C. nutans* at this site by removing all inflorescences after flowers had senesced but before they began to set seed.

### *Insect visitation*

*Visitation Rates:* In each plot at each sampling date I conducted 20-minute observations of the native plants. I observed half of the flowers in a plot in one 10-minute bout and the other half in a second 10-minute bout. For each observation bout, I recorded the number of flowers visited and divided this by the total number of flowers observed during that observation bout to calculate visitation rate (visits per flower per minute). For each date, I pooled data from the two observation bouts. I also recorded the type of flower visitor as *Bombus* species, solitary bee, Lepidoptera, Diptera, or other. As flower number can affect visitation rate, I also counted the number of *M. fistulosa* flower and *C. nutans* inflorescences.

I observed plots once every five to seven days during a 5-week period (Aug 5-Aug 31). Due to low flower numbers in some plots I did not observe all plots on all dates. All observations were carried out between 08:00 and 16:00 corresponding to peak insect activity (Cariveau *pers. obs.*).

*Interplant Movements:* Once every 5 to 7 days from August 16 to August 29, I conducted 20-minute observations of individual flower visitors in plots with *C. nutans* and recorded the sequence of plants they visited. Each time a flower visitor moved from one plant to another I recorded which species of plant the flower visitor departed and which species it visited next.

*Stigma pollen counts:* Once a week from the 15<sup>th</sup> to 29<sup>th</sup> of August, I collected up to 10 stigmas from each blooming *M. fistulosa* plant. For plants with fewer than 100 flowers, I collected only 10% of the stigmas to minimize the effect of stigma collection on overall seed set. I then dyed the stigmas using basic fuschin dye and counted the

number of *M. fistulosa* and *C. nutans* pollen grains under a compound microscope (Beattie 1971). I averaged the number of pollen grains per stigma per plant and then further averaged each plot to calculate the response variable of mean *M. fistulosa* and *C. nutans* pollen grains per stigma per plant for each plot on each date.

*Seed set:* Once a week from August 27<sup>th</sup> through October 26<sup>th</sup>, I collected all fruits from *M. fistulosa* as they ripened and counted all seeds within each fruit. For each collection date, I calculated the mean number of seeds produced per fruit for each plot by dividing the total number of seeds produced per plant by the total number of fruits for each plant. I then averaged the number of seeds per fruit for each plant to obtain a plot average for each collection date.

### Spatial Interactions

I examined the effect of distance from *C. nutans* patches on visitation rates to *M. fistulosa* from July 12 through July 21, 2006. This experiment was conducted at the Colorado State Forest Tree Nursery located at an elevation of 1560 meters in Fort Collins, Colorado. The nursery is comprised of tree rows, fallow fields, and open grass meadows that contained a mixture of native plants and the exotic *Bromus inermis*.

I constructed 8 plots in which I placed two potted *M. fistulosa* plants each at 0, 1, 5 and 15 m from a center point in which I added or removed *C. nutans*. I chose these distances based on a previous study that examined the distance at which flower visitors could distinguish between high and low nectar variants of a single plant species (Klinkhamer et al. 2001). Prior to placing potted native plants, I paired plants to maintain a consistent number of *M. fistulosa* flowers among all distances and then randomly

assigned paired plants to one distance point. Plots were at least 100 m from each other and there were few blooming flowers besides those blooming in the plots.

At each experimental plot I conducted one set of flower visitor observations with and one set without a patch of potted *C. nutans* in the center point. *Carduus nutans* patches consisted of 25 potted plants with a total of approximately 60 inflorescences (range: 48-65 flowers), well within the range of naturally occurring *C. nutans* populations (Cariveau *pers. obs.*). I randomly chose whether *C. nutans* would be added for the first or the second set of observations, and after the first observation bout, depending on treatment I either removed or added *C. nutans* and allowed the flower visitors to acclimate for 24 hours before conducting the second set of observations. *Monarda fistulosa* plants remained at each distance point for the entire experiment. Thus each plot was used for one set of observations with *C. nutans* in the center and one without *C. nutans*. I applied both treatments to each plot to minimize the effect of the spatial variation in flower visitor abundance and community composition (Williams et al. 2001).

*Observations:* Each morning I counted the total number of open flowers at each distance point within each plot and conducted one morning and one afternoon observation bout and pooled these data to obtain a value for visitation rate. Before the first set of observations, I randomly chose the order of the distances at which to observe the plants. I used this same order for the second set of observations. Observation bouts lasted between 15 and 40 minutes, depending on the available labor pool and were always conducted for the same duration for the before and after treatments of the same plot.

## Data Analysis

I used SAS v 9.1 to conduct all statistical analyses (SAS Institute 1999-2001). To examine the effect of *C. nutans* on visitation rate in the small plot and spatial experiments, stigma-pollen counts, and seed set in the small plot experiment, I used a mixed model ANOVA in the MIXED procedure. I used the arcsine (square root (x)) transformation for the visitation rate response variables. I used the  $\ln(x + 0.1)$  and  $\ln(x + 0.001)$  to transform pollen and seed set data. Fixed effects were treatment (presence or absence of *C. nutans*), date, and their interaction. Plot was a random effect in all models. *Carduus nutans* inflorescence number and *M. fistulosa* flower number were covariates. I used the number of *M. fistulosa* flowers and *C. nutans* inflorescences at each sampling date with exception of the seed set model. For the seed set model, I used the season-average flower and inflorescence number per plot as flower number at collection date did not correspond to flower number when individual fruits were open flowers. In the spatial experiment, distance was a categorical fixed effect. I included the order of *C. nutans* placement as half of the plots had *C. nutans* present in the center patch for the first observation while the other half had *C. nutans* in the second set of observations. When covariates and interactions were not significant I removed them from the final model.

I used a repeated measures design in all of these models to accommodate the multiple measurements on separate dates. I modeled correlations using the variance-covariance structure that resulted in the lowest Akaike's Information Criteria value. In the stigma counts for both *M. fistulosa* and *C. nutans* pollen grains, the autoregressive type 1 variance-covariance structure yielded the lowest AIC values. I used the autoregressive with heterogeneous variances type 1 variance-covariance structure in the

seed set analysis. The compound symmetric with heterogeneous variances and compound symmetric variance-covariance structures resulted in the lowest AIC value for the visitation rate analyses in the small plot and spatial experiment.

For each interplant switch executed by a flower visitor I calculated an expected probability that the switch would be to either a plant of the same species that it was currently visiting or the other species in the plot. I determined the expected probability by calculating the proportion of inflorescences available for each plant species. I then compared the total observed with the total expected switches using a Chi-squared test (FREQ procedure).

## **Results**

### Small-plot study

*Visitation rates:* I observed 297 flower visitors during 26 hours of observation. The majority of flower visitors were *Bombus* species (n = 111; 37%) and solitary bees (n = 103; 35%). Lepidoptera species made up 12% (n = 35) and Diptera and other flower visitors comprised 9% (n = 27) and 7% (n = 21). While *M. fistulosa* in the invaded plots had a higher visitation rate ( $0.064 \pm 0.01$  vs.  $0.040 \pm 0.008$ ) this difference was not significant ( $F_{1,54} = 0.85$ ,  $p = 0.36$ ; Fig. 1.1, Table 1.1). Visitation rate decreased over time ( $F_{4,54} = 24.77$ ,  $p < 0.001$ ; Fig. 1.1, Table 1.1). The interaction between date and treatment was not significant and was removed from the final model. *Monarda fistulosa* flower number ranged from 22 to 576 with a mean  $171 \pm 10.3$  and these flowers were contained in 4 to 29 inflorescences (mean =  $13.2 \pm 0.59$ ). Treatment plots contained from 1 to 22 *Carduus nutans* inflorescences with a mean of  $7.4 \pm 0.73$ . Despite the variation in *M.*

*fistulosa* flower number and *C. nutans* inflorescence number, neither of these covariates were significant and both were removed from the final model.

*Interplant movement:* Forty flower visitors made 106 interplant switches in 280 minutes of observation. *Bombus* species comprised 21 (53%) of the visitors while Lepidoptera and solitary bees made up 8 (20%) and 11 (27%) respectively. Flower visitors were more likely to move to *C. nutans* than expected and this was true whether moves were from *C. nutans* or *M. fistulosa*. Flower visitors departing from *M. fistulosa* switched to *C. nutans* 20 times yet I expected this to occur 14 times ( $\chi^2 = 4.22$ ,  $df = 1$ ,  $p = 0.04$ ; Fig. 1.2). Similarly, flower visitors leaving *C. nutans* moved to another *C. nutans* plant 49 times; I expected approximately 28 switches ( $\chi^2 = 25.50$ ,  $df = 1$ ,  $p < 0.001$ ; Fig. 1.2). Conversely, movements to *M. fistulosa* were lower than expected.

*Stigma-pollen counts:* *Monarda fistulosa* stigmas had more conspecific pollen when *C. nutans* was absent than when present ( $1.87 \pm 0.345$  vs.  $1.29 \pm 0.25$ ;  $F_{1,30} = 7.16$ ,  $p = 0.012$ ; Fig. 1.3A, Table 1.2). As the number of *M. fistulosa* flowers increased, the number of conspecific pollen grains increased as well ( $F_{1,30} = 19.43$ ,  $p < 0.001$ ; Fig. 1.3A, Table 1.2). Date was not significant ( $F_{1,30} = 0.93$ ,  $p = 0.41$ ; Table 1.2) yet the interaction between date and treatment was significant ( $F_{2,30} = 3.53$ ,  $p = 0.042$ ; Table 1.2) with the number of *M. fistulosa* pollen grains decreasing in invaded plots and increasing in non-invaded plots as the season progressed. The number of *C. nutans* inflorescences was not significant and I removed this from the final model.

*Carduus nutans* pollen was found in greater abundance on *M. fistulosa* stigmas in plots that contained *C. nutans* ( $F_{2,33} = 12.30$ ,  $p < 0.001$ ; Fig. 1.3B, Table 1.3). *Monarda fistulosa* stigmas in the presence of *C. nutans* had on average  $2.19 \pm 0.54$  *C. nutans*

pollen grains while stigmas of *M. fistulosa* not in the presence of *C. nutans* had  $0.36 \pm 0.07$  *C. nutans* pollen grains per stigma. The number of *C. nutans* pollen grains increased over time ( $F_{1,33} = 16.07$ ,  $p < 0.001$ ; Fig. 1.3B, Table 1.3), but the interaction between date and treatment was not significant and I removed this from the final model. I also removed the number of *M. fistulosa* flowers and *C. nutans* inflorescences from the final model. The mean number of seeds per fruit was greater in plots without *C. nutans* ( $0.29 \pm 0.043$  vs.  $0.20 \pm 0.028$ ;  $F_{1,66} = 1.96$ ,  $p = 0.167$ ; Table 1.4) although this effect was not significant. Seeds per fruit decreased over time ( $F_{7,66} = 2.70$ ,  $p = 0.016$ ; Table 1.4) and there was a significant positive effect of *M. fistulosa* flower number on seeds per fruit ( $F_{1,66} = 6.77$ ,  $p = 0.01$ ; Table 1.4). The interaction between date and treatment and the number of *C. nutans* were not significant and I removed these from the final model.

### Spatial Experiment

In total 617 flower visitors were observed on *Monarda fistulosa* flowers. *Bombus* species made up 41% of the visitors ( $n = 251$ ), solitary bees comprised 30% ( $n = 188$ ) and 21% were Lepidoptera ( $n = 133$ ). Six percent ( $n = 35$ ) were from *Apis mellifera*, and Diptera and other flower visitors each comprised 2% ( $n = 10$ ). Overall, the presence vs. absence of *C. nutans* was significant ( $F_{1,46} = 8.64$ ,  $p = 0.005$ ; Fig. 1.4, Table 1.5) with more flowers visited in the absence of *C. nutans*. The interaction between distance and treatment was only marginally significant ( $F_{3,46} = 2.16$ ,  $p = 0.105$ ; Fig. 1.4, Table 1.5). As this was the main hypothesis in this experiment, I continued to investigate differences at each distance for the treatment effect. *Monarda fistulosa* flowers at a distance of 0 and 15 meters did not experience a reduction in visitation when *C. nutans* was present in the center patch ( $t = -0.19$ ,  $df = 46$ ,  $p = 0.85$  and  $t = 0.72$ ,  $p = 0.47$ ; Fig. 1.4), while *M.*

*fistulosa* flowers at 1 and 5 meters received fewer visits in the presence of *C. nutans* ( $t = 2.28$ ,  $df = 46$ ,  $p = 0.027$  and  $t = 3.06$ ,  $df = 46$ ,  $p = 0.004$ ; Fig. 1.4). Visitation rate decreased over time ( $F_{5,46} = 2.63$ ,  $p = 0.036$ ; Table 1.5). Distance in the absence of its interaction with treatment was not significant in the model ( $F_{3,46} = 0.46$ ,  $p = 0.71$ ; Table 1.5). The number of *M. fistulosa* flowers ranged from 178 to 703 over all distances with a mean of  $327 \pm 4.2$ . The number of *M. fistulosa* flowers, *C. nutans* inflorescences, and the order of *C. nutans* placement were not significant and I removed these covariates from the final analysis.

## Discussion

Plants may compete through flower visitors by decreasing visitation rate, transferring heterospecific pollen grains, or a combination of both processes. I found evidence that the presence of an invasive plant influences flower visitor behavior in relation to a native plant. Most importantly, I found that decreases in visitation rate occurred when *M. fistulosa* was spatially separated from *C. nutans* but not when the plants were in close proximity. While the quantity of visits was not significantly different when *M. fistulosa* was in close proximity to *C. nutans*, the quality of these visits may be reduced in the presence of *C. nutans*. In the small plot experiment, flower visitors transferred more heterospecific and fewer conspecific pollen grains when *M. fistulosa* was in the presence of *C. nutans*. Multiple studies have tested for competition through both heterospecific pollen transfer and decreases in visitation rate (e.g. Campbell and Motten 1985, Brown et al. 2002) and these results add to the growing knowledge of how native and invasive plants interact through flower visitors (see Bjercknes et al. 2007).

Furthermore, this study provides evidence that interspecific competition among plants may occur at spatial scales much greater than the zone of rhizosphere overlap.

I suspect that I did not detect a difference in visitation rate to *M. fistulosa* in either the small plot experiment or in close proximity to *C. nutans* in the spatial experiment because although floral visitors were highly attracted to *C. nutans*, some temporarily switched to *M. fistulosa* during their foraging bout. These ‘spill-over’ visits are not likely to be of high quality as conspecific pollen increased and heterospecific pollen decreased in the presence of *C. nutans*. Results from the spatial experiment demonstrated that the differences in visitation rate to *M. fistulosa* were greatest when they were separated from *C. nutans* by 1 to 5 m. It is likely that there are two processes operating simultaneously to produce this pattern: Competition for flower visitor preference and facilitation due to the large central patch of *C. nutans* flowers coupled with switching behavior. These data suggest that competition is operating on a larger spatial scale (1 – 5 m) than facilitation (< 1m). When combined, these two processes produce the pattern of no change in visitation for plants immediately adjacent to the *C. nutans* patch and at distances greater than the range of competitive interaction, but a significant drop off in visits for plants at intermediate distances. This may result in a decrease in heterospecific pollen transfer as spatial separation increases. Although this pattern is consistent with the results from the small plot experiment, confirmation of this scenario would require either direct observation of switching behavior or measurement of heterospecific pollen transfer. Given the large spatial extent and design of the experiment (repeated observations of the same array with and without *C. nutans*) I was unable to collect these data.

*Monarda fistulosa* seed set was 31% greater in the absence of *C. nutans* in the small plot experiment but it was not significant. Although there was a trend towards a reduction in visits in the small plot experiment, it may not have been enough to lead to a decrease in seed set. In the spatial experiment I found decreases in visitation to *M. fistulosa* in the presence of *C. nutans* at 1 and 5 meters. I suspect that these large differences would result in changes in seed set; however, I did not measure reproductive success in this experiment. *Monarda fistulosa* flowers, like other members of the Lamiaceae, each contain four ovules and seed set was quite low in small plot experiment. This may be due to the low flower density in the plots. Plants in small plots often have low visitation rates, and increased pollen limitation and increasing conspecific plant number can increase visitation rate and seed set (Knight 2003). In the small plot study, I found some support for this pattern as plots with more *M. fistulosa* flowers had more conspecific pollen grains and seeds per fruit. However, I found no affect of *M. fistulosa* flower number on visitation rate. Natural populations of *M. fistulosa* vary in size and the numbers I used in these experiments are at the low range of what occurs in natural settings (Cariveau *pers. obs.*) The presence of heterospecific plants can also increase the overall flower density in a patch and lead to an increase in the reproductive success of a second species (Moeller 2004) and this can occur despite heterospecific pollen transfer (Feldman et al. 2004). However, *C. nutans* flower number was not a significant factor in any of the models. The densities of *C. nutans* flowers in the small plot experiment were at the low end of that seen in natural, invaded populations and the magnitude and type of interactions found in this study may change at larger densities of the invasive plant (Rathcke 1983).

The presence of heterospecific pollen grains can also reduce seed set if it causes premature stigmatic closure (Waser and Fugate 1996) or chemically impedes germination of conspecific pollen (e.g. Murphy and Aarsen 1995). *Carduus nutans* pollen may not have these effects on *M. fistulosa* stigmas and pollen. Spatial occlusion was also unlikely as *M. fistulosa* stigmas had ample space for more pollen (Cariveau *pers. obs.*). While flower visitors did switch from *C. nutans* to *M. fistulosa*, these switches were lower than expected and this likely kept the number of *C. nutans* pollen grains on *M. fistulosa* stigmas low.

An important question remains unanswered in this and other studies of flower visitor-mediated interactions between native and invasive plants: Are the effects of exotic species different in magnitude from those of other natives? Many exotic, invasive plants have large floral displays (Bjerknes et al. 2007). Further, other work has demonstrated that plants attacked by herbivores have smaller floral displays and reduced floral rewards (Strauss et al. 1996, Lehtila and Strauss 1997). Because many of the herbivores of exotic plants are less abundant in a plant's invaded range (Andres and Goeden 1971), invasive plants may suffer less damage to floral displays or rewards and be able to allocate more resources to flower visitor attraction than native plants. This idea remains untested.

Exotic plant invasions are common phenomena that potentially lead to decreases in native plant diversity. However, research that examines the mechanisms causing this decline are lacking (Levine et al. 2003). Indirect competition through flower visitors is a viable mechanism that could result in negative impacts to native plants. These results suggest that competition among plants may occur at larger spatial scales than those considered in traditional plant competition studies. It is important to consider multiple

mechanisms of competition as well as spatial context when examining the effects of exotic plants on native plants.

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Table 1.1. Repeated measures ANOVA examining the response of *Monarda fistulosa* visitation rate to date and *Carduus nutans* presence.

Variable	Effect	DF	F	p
Visitation Rate	Date	4, 54	24.77	< 0.001
	<i>C. nutans</i> presence	1, 54	0.85	0.360

Table 1.2. Repeated measures ANOVA examining the response of *Monarda fistulosa* pollen grains per *M. fistulosa* stigma to *Monarda fistulosa* flower number, date, *Carduus nutans* presence and the interaction between *Carduus nutans* presence and date.

Variable	Effect	DF	F	p
<i>M. fistulosa</i> pollen	Date	2, 30	0.93	0.410
	<i>C. nutans</i> presence	1, 30	7.16	0.012
	<i>C. nutans</i> x date	2, 30	3.53	0.042
	<i>Monarda fistulosa</i> flower	1, 30	19.43	<0.001

Table 1.3. Repeated measures ANOVA examining the response of *Carduus nutans* pollen grains per *Monarda fistulosa* stigma to date, *Carduus nutans* presence and their interaction.

Variable	Effect	DF	F	p
<i>C. nutans</i> pollen	Date	2, 33	12.30	< 0.001
	<i>C. nutans</i> presence	1, 33	16.07	< 0.001

Table 1.4. Repeated measures ANOVA examining the response of *Monarda fistulosa* seeds per fruit to *Monarda fistulosa* flower number, date, *Carduus nutans* presence and the interaction between date and *Carduus nutans* presence.

Variable	Effect	DF	F	p
Seeds per Fruit	Date	7, 66	2.70	0.016
	<i>C. nutans</i> presence	1, 66	1.96	0.167
	<i>M. fistulosa</i> flowers	1, 66	6.77	0.011

Table 1.5. Repeated measures ANOVA examining the response of *Monarda fistulosa* visitation rate to date, distance from *Carduus nutans* patch, presence of *Carduus nutans* and the interaction between distance and *Carduus nutans* presence.

Variable	Effect	DF	F	p
Visitation Rate	Date	5, 46	2.63	0.036
	<i>C. nutans</i> presence	1, 46	8.64	0.005
	Distance from <i>C. nutans</i>	3, 46	0.46	0.710
	Presence x Distance	3, 46	2.16	0.105

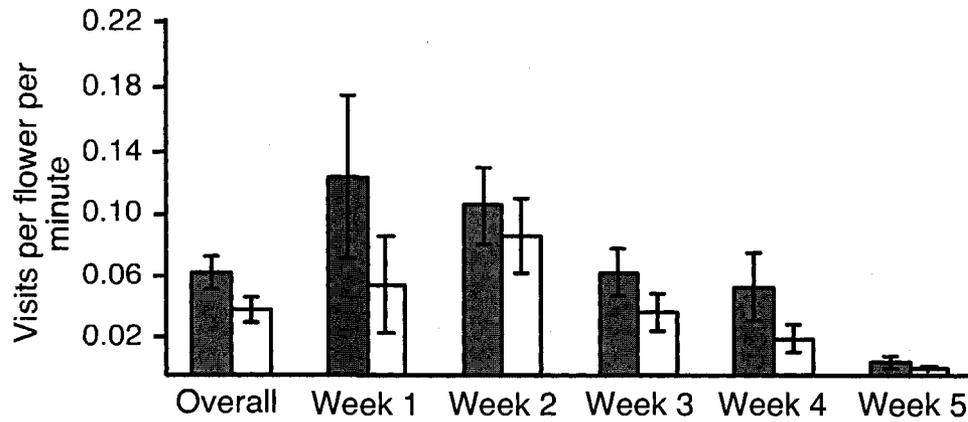


Figure 1.1. Visits per *Monarda fistulosa* flowers per minute by date. Solid bars represent visitation rate without *Carduus nutans*. Open bars indicate the *Carduus nutans* treatment. Analysis was conducted using the arcsine (square root) transformation. Data presented are non-transformed means. Error bars represent  $\pm 1$  standard error.

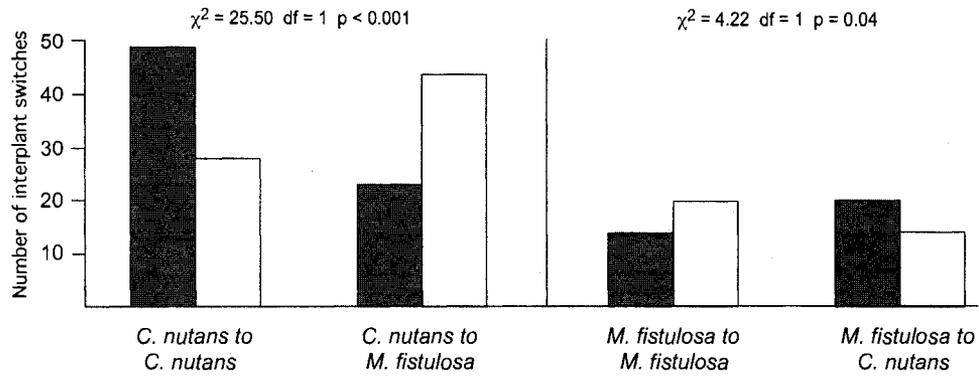


Figure 1.2. Observed and expected movements between plant species by flower visitors in the presence of *Carduus nutans*. Solid bars indicate the observed number of switches. Open bars represent the expected number of switches.

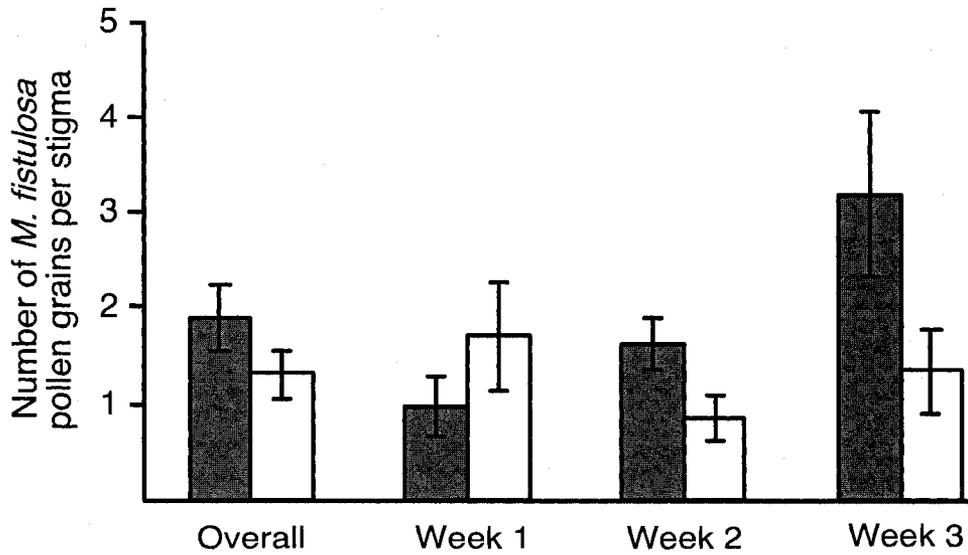


Figure 1.3A. Mean number of *Monarda fistulosa* pollen grains per stigma without and with the presence of *Carduus nutans*. Solid bars represent number of *Monarda fistulosa* pollen grains per stigma without *Carduus nutans*. Open bars indicate the *Carduus nutans* treatment. Analysis was conducted using the  $\ln(x + 0.1)$  transformation. Data presented are non-transformed means. Error bars represent  $\pm 1$  standard error.

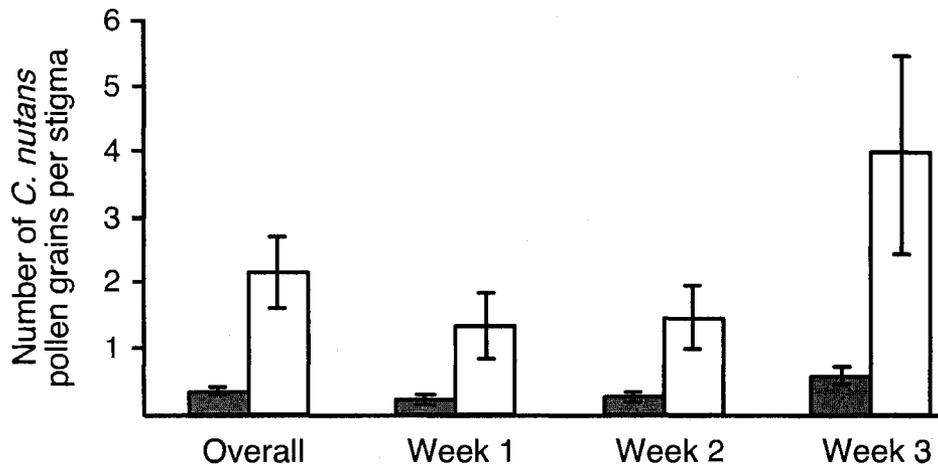


Figure 1.3B. Mean number of *Carduus nutans* pollen grains per stigma without and with the presence of *Carduus nutans*. Solid bars represent number of *Carduus nutans* pollen grains per stigma without *Carduus nutans*. Open bars indicate the *Carduus nutans* treatment. Analysis was conducted using the  $\ln(x + 0.1)$  transformation. Data presented are non-transformed means. Error bars represent  $\pm 1$  standard error.

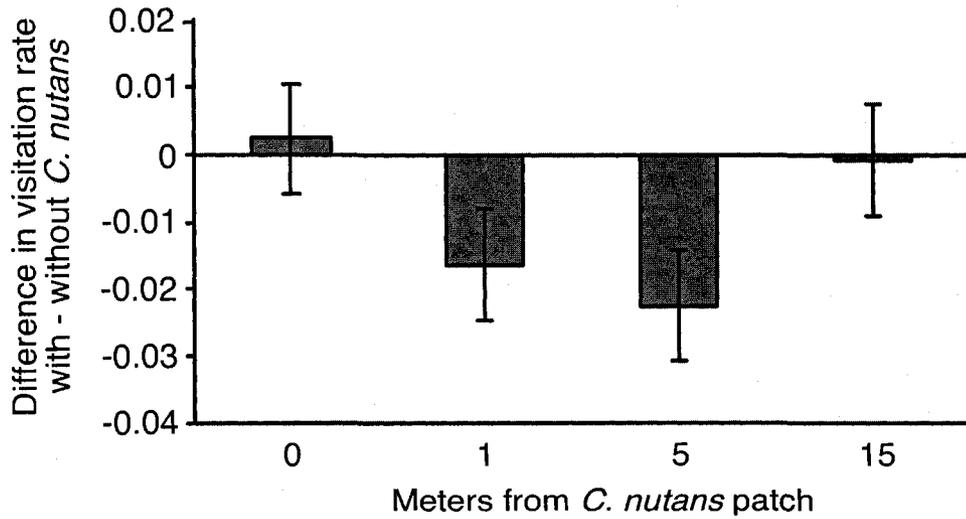


Figure 1.4. Difference in visitation rate at each distance point. Negative values indicate lower visitation rates in the presence of *Carduus nutans*. Analysis was conducted using the arcsine (square root) transformation. Data presented are non-transformed means. Error bars represent  $\pm 1$  standard error.

## CHAPTER TWO

FLOWER VISITOR-MEDIATED INTERACTIONS BETWEEN THE EXOTIC PLANT  
MUSK THISTLE (*CARDUUS NUTANS*) AND THE NATIVE PLANT COMMON  
HAREBELL (*CAMPANULA ROTUNDIFOLIA*).

## Abstract

Invasions of exotic plants are a ubiquitous process that can have dramatic effects on the fitness and population dynamics of native plants. These effects can occur through a variety of pathways, including altering interactions among native plants and their mutualists. For example, exotic and native plants often bloom simultaneously and share flower visitors, and this may lead to competition for pollination services. Exotic plants may be more attractive to flower visitors, which could reduce visitation rate to and potentially seed set of native plants. Flower visitors may also transfer exotic pollen to native plant stigmas, which can impede the ability of conspecific pollen to fertilize ovules. To determine if and how an exotic plant interacts with a native plant through flower visitors, I addressed the following questions: 1) Does the presence of the exotic plant musk thistle (*Carduus nutans*) affect visitation rates with respect to the native plant, common harebell (*Campanula rotundifolia*)? 2) In the presence of the exotic, is there a difference in the quantity of conspecific and heterospecific pollen grains deposited on common harebell stigmas and seeds per common harebell fruit? I constructed experimental arrays of potted common harebell with half of the arrays containing musk thistle. For common harebell plants, I recorded visitation rates for solitary bees, and *Bombus* species, pollen deposition rates, flower production, and seed set. I found that in arrays with the exotic plant, visitation rate of solitary bees were reduced by 80%. Musk thistle presence reduced visitation rate of *Bombus* species by 40% but the effect was not significant. However, conspecific pollen deposition and seeds per fruit were not different with and without musk thistle. I suggest that the difference in visitation rate yet lack of effect on pollen deposition and seeds per fruit may have occurred because the most

efficient pollinators (*Bombus* species) were not affected by musk thistle presence. In addition, plants in invaded plots retained flowers for longer periods of time suggesting longer stigma receptivity that enabled compensatory levels of pollen transfer.

Competition through heterospecific pollen transfer did not occur in this study. Flower visitors did not switch between the two species and I found no musk thistle pollen grains on common harebell stigmas.

## **Introduction**

Invasive plants occur throughout terrestrial ecosystems and their presence often results in negative consequences for native plant populations and communities. Most studies that have addressed the interaction between invasive and native plants have considered competition for water, nutrients, or light (Levine et al. 2003). However, a number of recent studies have found that native and invasive plants also interact through shared mutualists (Brown et al. 2002, Bray et al. 2003, Traveset and Richardson 2006). For example, multiple plant species share flower visitors (Waser et al. 1996) and the presence of an invasive plant may influence flower visitor behavior with respect to the native plant. This change in behavior may then affect the quantity and/or quality of pollen reception. If an invasive plant attracts more flower visitors than a sympatric, simultaneously blooming native, the native plant may experience a reduction in flower visits. This may subsequently result in a reduction of conspecific pollen grains reaching native plant stigmas. Flower visitors may also switch between native and invasive plants within the same visitation bout and transfer invasive plant pollen to native plant stigmas. This transfer of heterospecific pollen may impede conspecific pollen from fertilizing ovules (Brown and Mitchell 2001, Waser and Fugate 1996). Flower visitors that move between species in a single foraging bout may also lose pollen on interspecific plants potentially reducing the overall availability of conspecific pollen (Campbell and Motten 1985). Conversely, the presence of one plant may also lead to an overall increase in flower visitors, and this augmentation may lead to an increase in visits among other conspecific plants (e.g. Ghazoul 2006).

Many invasive plants have large, conspicuous floral displays and possess an inflorescence architecture that allows for visitation by multiple species of flower visitors (e.g. Chittka and Schurkens 2001, Ghazoul 2002). Therefore, exotic plants are likely to interact, potentially quite strongly, with native plants through flower visitors. The few studies that have addressed this interaction found a variety of effects including competitive (Grabas and Lavery 1999, Chittka and Schurkens 2001, Brown et al. 2002, Bartomeus et al. 2008), facilitative (Grabas and Lavery 1999, Moragues and Traveset 2005, Lopezaraiza-Mikel et al. 2007, Bartomeus et al. 2008), and neutral impacts (Ghazoul 2004, Jones 2004, Moragues and Traveset 2005, Totland et al. 2006, see Bjerknes et al. 2007 for review).

The purpose of this study was to examine how an exotic, invasive plant affects the visitation rate, pollen deposition, and seed set of a native plant. To address this question, I constructed 10 arrays with only the native plant, common harebell (*Campanula rotundifolia* L., Campanulaceae) and 10 arrays that included common harebell as well as the exotic plant (*Carduus nutans* L., Asteraceae). Using this experimental design, I addressed two questions: 1) Does the presence of the exotic plant musk thistle affect the behavior of flower visitors with respect to the native plant common harebell?; and 2) In the presences of the exotic, is there a difference in the quantity of conspecific and heterospecific pollen grains deposited on common harebell stigmas and seeds per harebell fruit?

## Methods

### Study Species

Common harebell is a perennial, herbaceous forb native in much of North America (USDA, NRCS 2008). It reproduces solely through seed production and is self-compatible (Bingham 1997). Seed set is increased in the presence of flower visitors, and the number of seeds per flower is limited by pollen deposition at other sites in Colorado (Bingham 1997). Common harebell produces perfect, actinomorphic, bell-shaped flowers with up to 30 concurrent 1 x 3 cm flowers on each plant. Musk thistle is native to northern Asia and Europe (Whitson et al. 2002). It was introduced into North America in the early 1800s and is invasive in Australia, New Zealand, North America, and South America (USDA, NRCS 2008, Gobbi et al. 1995, Shea et al. 2005). It is biennial and produces a 1-2 meter flowering stalk with up to 5 simultaneously blooming inflorescences that are 3-5 cm in diameter (Whitson et al. 2002). Common harebell is primarily visited by bees in Colorado (Bingham and Orthner 1998). Many bee species also visit musk thistle as do numerous Lepidoptera, Coleoptera, and Diptera species (Cariveau *unpublished data*). Despite differences in inflorescence morphology, musk thistle and common harebell share many of the same hymenopteran flower visitors including Apidae (*Bombus* species), Megachilidae, and Halictidae (Cariveau *unpublished data*).

### Study Site

I conducted this study in the Arapahoe-Roosevelt National Forest in Larimer County, Colorado, USA at approximately 2500 m. The site is forested and dominated by *Pinus ponderosa* Douglas ex. Louden (Pinaceae), *Pinus contorta* C. Lawson (Pinaceae),

and *Pseudotsuga menziesii* (Mirb.) Franco (Pinaceae). Interspersed throughout are openings that contain a number of grass and flowering forb species. I constructed the experimental arrays in these openings (see below). Few common harebell individuals existed at the site and the nearest known musk thistle population was over 2 km away.

### Arrays

To examine the effects of musk thistle on visitation rates of potential pollinators to common harebell, I created experimental arrays using potted plants. Plants may interact through resources; I therefore used potted plants to isolate treatment effects to interactions between plants through flower visitors.

I purchased common harebell from a native plant nursery in Colorado (Rocky Mountain Native Plants Company; Rifle, Colorado, USA) and collected musk thistle rosettes from a location near the study site. I transplanted all plants into 8 L pots and placed them outside at the Colorado State University campus until the start of the experiment. As flowers that were blooming while at Colorado State University campus were not in their respective treatment arrays the seed production and pollen deposition measurements would not reflect musk thistle presence versus absence. Therefore, I removed all flowers that bloomed before the start of the experiment.

Ten of the arrays were invaded and consisted of 4 musk thistle and 4 common harebell. The 10 native-only arrays consisted of 6 common harebell. The original intent of this experiment was to include a second native species (bee balm (*Monarda fistulosa*: Lamiaceae) so that non-invaded plots contained 6 of each native (12 total) and invaded plots included 4 of each native and 4 musk thistle (12 total). However bee balm failed to bloom and instead of modifying the experiment while it was occurring, I used flower

number as a covariate in the models (see Statistical Analyses). Arrays remained in the field for the entire length of the experiment. I randomly assigned arrays to either musk thistle presence or absence. To prevent the spread of this invasive plant, I removed all musk thistle inflorescences after flowering but before seed set. Within arrays, plants were separated by 1 m and I separated arrays by at least 100 m. I removed all flowers other naturally occurring species within 1 m of the arrays to reduce variation in floral background among arrays. I kept plants well-watered and added fertilizer monthly (24-8-16: Scotts Miracle-Gro All Purpose Plant Food, Marysville, Ohio, USA).

*Visitation Rates:* Once every 5 to 7 days from August 6 to August 24, I conducted two fifteen-minute observation bouts at each array. During an observation bout, I counted the number of common harebell flowers visited. A visit was recorded when a flower visitor entered the flower and thus contacted the reproductive parts. After each observation, I calculated visits per flower per minute (herein visitation rate). I also recorded morpho-taxon of each flower visitor as solitary bee (included members of the following families: Megachilidae, Halictidae, Andrenidae) or *Bombus* (Apidae) species. I observed only those arrays that contained greater than 10 flowers, thus not all arrays were observed on all dates.

*Flower number:* To determine whether common harebell flower number influenced visitation rate, I counted the total number of flowers per array and used this measurement as a covariate in the models. After reviewing the data, I found that while visitation rates were higher in arrays without musk thistle, pollen deposition was not affected (See Results). One way that common harebell could compensate for a reduction in visitation would be to retain flowers until they are fertilized. Plasticity in floral

longevity is known for other plants and has been suggested for common harebell as well (Giblin 2005). To examine this, I used the number of flowers per common harebell plant per date. I suspected that in invaded arrays, common harebell plants maintained flowers for a longer period of time and had more flowers per plant than common harebell in non-invaded arrays.

*Stigma Pollen Counts:* Once a week from August 10 to September 11, I counted pollen loads on common harebell plants by collecting one stigma from a pistillate flower from each plant, mounting the stigma on a microscope slide, staining it with basic fuchsin dye, and then counting grains under 40x magnification (Beattie 1971). Removal of stigmas likely decreased seed set of the flowers in which stigmas were removed. Therefore, I did not remove stigmas from plants that had fewer than 5 flowers to minimize the proportion of flowers damaged. Using reference slides of anthers of each plant, I was able to distinguish musk thistle pollen from common harebell pollen.

*Seed Set:* I collected all common harebell fruits as they matured but before dehiscence and counted the number of seeds per fruit at each sampling date. I then calculated the mean number of seeds per fruit for each plant and took an average of plants within the array for a per array average at each sampling date.

### Data Analyses

All statistical tests were conducted using Proc Mixed (SAS v9.1; SAS Institute). I used a repeated measures design as it allowed me to record multiple measurements on the same array on separate dates. In all of the models, array (and not individual plant) is the unit of replication. I used the variance-covariance structure that resulted in the lowest Akaike's Information Criterion (AIC) value to model correlated errors of measurements

within arrays. In addition, I removed interactions and covariates from the final models when not significant ( $p > 0.1$ ).

*Visitation:* To determine whether musk thistle presence was affecting visitation rate, I designated date and the presence of musk thistle as fixed effects and array as a random effect. The daily number of flowers per array was a covariate. The number of visits per flower per minute (arc-sine square root transformed) was the response variable. I used this design to test the influence of musk thistle presence on each of the three categories of visitors: all taxa, solitary bees, and *Bombus* species. In the all taxa visit model, the autoregressive type 1 variance-covariance structure resulted in the lowest AIC value. The autoregressive type 1 with heterogeneous variances variance-covariance structure resulted in the lowest AIC value for the solitary bees and *Bombus* species visitation rate.

*Flower number:* To test whether musk thistle presence influenced the daily number of flowers per plant, I used musk thistle presence and date as fixed effects and array as a random effect. As I was explicitly interested in determining how the progression of date affected the number of flowers, I designated date as a continuous variable. The response variable was the mean number of flowers per plant per day ( $\ln(x + 1)$  transformed). The autoregressive type 1 variance-covariance structure resulted in the lowest AIC value.

*Pollen:* To assess whether the presence of the exotic plant influenced pollen deposition, I selected date and musk thistle presence as fixed effects and array as a random effect. The total number of common harebell flowers in each array at each sampling date was a covariate. The response variable was mean number of conspecific

pollen grains per stigma per plant ( $\ln(x + 1)$  transformed). The autoregressive type 1 with heterogeneous variances variance-covariance structure resulted in the lowest AIC

*Seed set:* In the pollen model, date and the presence of musk thistle were fixed effects and array was a random effect. I used the overall season mean number of common harebell flowers as a covariate as I did not record the date at which each individual fruit was a flower. I also included the overall season mean visitation rate of *Bombus* species and solitary bees. The response variable was the mean number of seeds per fruit per plant ( $\ln(x + 1)$  transformed). The autoregressive type 1 with heterogeneous variances variance-covariance structure resulted in the lowest AIC value.

## Results

*Visitation: Visitation All Species:* Visitation rate was higher for all flower visitors combined in arrays without musk thistle than in the arrays with musk thistle ( $0.016 \pm 0.0023$  vs.  $0.006 \pm 0.0015$ ,  $F_{1,33} = 20.79$ ,  $p < 0.001$ ; Fig 2.1A, Table 2.1A). Visitation rate decreased as the number of common harebell flowers increased but it was not significant ( $F_{1,33} = 2.73$ ,  $p = 0.062$ ). I found no evidence for an effect of date ( $F_{1,33} = 1.45$ ,  $p = 0.25$ ). The interaction of treatment and date was not significant and I removed it from the final model. *Visitation Solitary Bees:* The visitation rate of solitary bees was lower in arrays with musk thistle than in those without ( $0.01 \pm 0.0021$  vs.  $0.002 \pm 0.0001$ ;  $F_{1,35} = 30.19$ ,  $p < 0.001$ , Fig. 2.1B, Table 2.1B). It also decreased with date but it was not significant ( $F_{1,35} = 2.37$ ,  $p = 0.087$ ). Neither the interaction between date and musk thistle presence nor the number of common harebell flowers was significant and I removed these from the final model. *Visitation *Bombus* species:* *Bombus* species also tended to visit more flowers in the arrays without musk thistle ( $0.005 \pm 0.0015$  vs.  $0.003 \pm 0.0012$ ; Fig. 2.1C, Table

2.1C) but this was not significant ( $F_{1,32} = 2.61$ ,  $p = 0.116$ ; Fig. 2.1C, Table 2.1C). Date and the interaction between date and treatment were significant ( $F_{3,32} = 4.29$ ,  $p = 0.012$ ;  $F_{3,32} = 3.73$ ,  $p = 0.048$ ; Fig. 2.1C, Table 2.1C) with an increase in visits as the season progressed. However, the effect of treatment was not significant at any of the dates. The number of common harebell flowers was not significant, and I removed this covariate from the final model.

*Stigma Pollen Counts:* I did not detect a difference in the number of common harebell pollen grains on common harebell stigmas in arrays with and without musk thistle ( $63.24 \pm 4.00$  vs.  $56.68 \pm 3.69$ ;  $F_{1,56} = 0.10$ ,  $p = 0.75$ , Fig. 2.2, Table 2.2). I did find that pollen deposition increased with date ( $F_{5,56} = 8.81$ ,  $p < 0.0001$ ). The interaction between date and treatment was significant ( $F_{5,56} = 2.46$ ,  $p = 0.044$ ). The number of common harebell flowers was not significant and I removed it from the final model. After examining 242 stigmas, I found no musk thistle pollen on any common harebell stigmas.

*Flower Number:* Analysis of the weekly census data indicated that the overall number of flowers per plant was not affected by treatment ( $F_{1,85} = 1.02$ ,  $p = 0.315$ ; Fig. 2.3, Table 2.3). However, the interaction between date and treatment was significant with the number of flowers per plant being higher in the presence of musk thistle at later dates ( $F_{1,85} = 6.25$ ;  $p = 0.014$ ; Fig. 2.3, Table 2.3). The overall relationship between date and flower number was a quadratic function ( $F_{1,85} = 6.03$ ;  $p = 0.016$ ; Fig. 2.3, Table 2.3) but the effect on the model was small. There was no interaction between the quadratic term and musk thistle presence and this was dropped from the analysis.

*Seed Set:* There was no significant effect of musk thistle on the number of common harebell seeds per fruit ( $64.26 \pm 6.9$  vs.  $55.58 \pm 6.1$ ,  $F_{1,85} = 0.05$ ;  $p = 0.82$ ; Fig.

2.4, Table 2.4). Seeds per fruit increased with the overall season mean number of common harebell flowers per array ( $F_{1,85} = 8.40$ ;  $p = 0.005$ ; Fig. 2.4, Table 2.4) and over time ( $F_{7,85} = 5.57$ ;  $p = <0.001$ ; Fig. 2.4, Table 2.4). The covariate of the season mean *Bombus* visitation rate was marginally significant ( $F_{1,85} = 3.76$ ;  $p = 0.056$ ; Fig. 2.4, Table 2.4) with an increase in *Bombus* visitation rate associated with an increase in seeds per fruit. Neither solitary bee visitation rate nor the interaction between date and treatment were significant and I removed these factors from the final model.

### **Discussion**

In addition for competing for resources such as light, nutrients, and water, plants may also compete through flower visitors. As they often have large floral displays, invasive plants have the potential to draw away flower visitors from native plants. I found evidence for this pattern in this study: common harebell that did not occur with musk thistle had more flowers visited than those occurring with this invasive plant. However, neither the quantity of pollen deposited nor the number of seeds produced per fruit was affected by musk thistle presence. In addition, there was no evidence that musk thistle competes with common harebell through heterospecific pollen transfer as I found no musk thistle pollen grains on common harebell stigmas. In the following discussion, I address two main questions: 1) Why were there fewer visits to common harebell in the presence of musk thistle but no concomitant difference in conspecific pollen deposition or seed set?; and 2) Why did heterospecific pollen transfer not occur between these two plant species?

I suggest three hypotheses to explain why a reduction in visitation rate did not lead to a concomitant reduction in seed set: 1) Seed set was limited by resource

availability and not pollen deposition. 2) Musk thistle presence only affected visitation of inefficient pollinators. 3) Characteristics of the mating system such as floral longevity and the ability to self-pollinate may buffer the negative effects of a reduced visitation rate. Sexual selection theory predicts that resources will determine female fitness while the number of matings (i.e. visits) will have little effect (Bateman 1948). Resource availability has been shown to limit female reproductive success (Campbell and Halama 1993). However, seed set in many plants is limited by the number of pollen grains deposited (Burd et al. 1994, Ashman et al. 2004, Knight et al. 2005). In two other studies of naturally-occurring common harebell in Colorado, plants often produced up to 140 seeds per fruit, almost 3 times the amount seen in this study, indicating that seed set of plants in this study was lower than those found in natural populations (Bingham 1997, Giblin 2005). While I kept plants well watered and fertilized, I cannot rule out that seed set was constrained by available resources. However, as I did not conduct pollen supplementation experiments, I am unable to disentangle the effect of pollen versus resource limitation on seed set.

Musk thistle presence may have only reduced visits of inefficient pollinators to common harebell. Flower visitors vary in the effectiveness of pollination (Schemske and Horvitz 1988, Wilson and Thomson 1991, Johnson et al. 1995). I found that averaged over all dates, solitary bees visited 80% fewer common harebell flowers when musk thistle was present. *Bombus* species visits tended to be lower in musk thistle-invaded arrays as well but the reduction was approximately 40% and was not significant. The overall reduction in visitation rate may have been inconsequential for seed set as the reduction was primarily pronounced for solitary bees that may be inefficient pollinators.

In Colorado, Bingham and Ranker (2000) found that *Bombus* species transferred more pollen grains per visit to common harebell than other bee species. In a study of the congener *Campanula americana*, Johnson et al. (1995) found that solitary bees in the genus *Halictus* were inefficient pollinators as they visited primarily male-phase flowers to collect and consume pollen. In contrast, members of the *Bombus* genus visited both male and female phase flowers while foraging primarily on nectar and were efficient pollinators. I found evidence for this as seeds per fruit was positively associated with season mean *Bombus* visitation rate while solitary bee visitation rate had no effect.

Common harebell may have compensated for fewer visits by increasing the amount of time each flower remained open. Weekly census data showed that individual common harebell plants in invaded arrays had more flowers as date increased while flowers per plant declined in non-invaded arrays. As the number of fruits per plant was not different in the absence vs. presence of musk thistle ( $19.4 \pm 4.4$  vs.  $19.38 \pm 5.5$ ;  $t_{17} = -0.005$ ,  $p = 0.996$ ), the best explanation for the pattern is that common harebell flowers in invaded arrays remained open longer than those in arrays where musk thistle was absent. This plasticity in flower retention time is likely an adaptation to ensure reception of sufficient pollen receipt, removal, or both. Common harebell may have retained flowers longer in the arrays with musk thistle, and this may have resulted in the similar quantity of conspecific pollen found on common harebell stigmas. In a study of common harebell in Colorado, Giblin (2005) found that at one site, experimentally delaying flower visitors increased the duration of the pistillate phase. Additionally, there is some evidence that harebell is autogamous in some populations (Bingham 1997), suggesting harebell may be able to compensate for decreased visits through self-pollination. However, this is not

always the case (Nyman 1992, Giblin 2005). These results suggest that characteristics of the mating system such as floral longevity and self-fertilization may buffer some plants from reduced visitation rates due to the presence of an invasive plant.

Flower visitors can transfer heterospecific pollen grains that may impede conspecific pollen from siring ovules. Two observations strongly suggest that this did not occur in this experiment. First, out of 1365 minutes, I observed only one flower visitor switching from common harebell to musk thistle. Despite sharing species of flower visitors, I was confident that flower visitors rarely switch between musk thistle and common harebell within a single feeding bout. Second, after examining 242 common harebell stigmas and counting over 16000 pollen grains, I found no musk thistle pollen grains on common harebell stigmas. Furthermore, I counted only 3 pollen grains from other plant species. This lack of switching behavior and heterospecific pollen deposition is likely due to the large discrepancies in floral morphology between these two species. Musk thistle is much taller and the flowers are tightly clumped into 3-5 cm inflorescences, while common harebell has 1 x 3 cm widely spaced bell-shaped flowers. Other work has demonstrated that during a given foraging bout, flower visitors will not switch between species that are highly divergent in floral traits (Gegear and Lavery 2005). In a separate study, the native plant bee balm that occurred with musk thistle had numerous musk thistle pollen grains deposited on their stigmas (Cariveau and Norton *submitted*). The floral morphology of musk thistle and bee balm are much more similar than musk thistle and common harebell. Understanding how similarities and discrepancies of floral displays drive the interaction among plants through flower visitors

may allow for the prediction of the type and magnitude of this interaction (Sargent and Ackerly 2008).

Despite the ubiquity of and negative consequences imposed by invasive plants, relatively few studies have examined the mechanisms by which invasive plants affect native plant populations and communities (Levine et al. 2003). Studies addressing interactions between native and invasive plants through flower visitors are rare and the results have shown negative, neutral, and positive interactions. The results of this study and others (Muñoz and Cavieres 2008, see Bjercknes et al. 2007 for review) suggest that although interactions may be common, negative impacts may occur only in specific cases. Understanding how traits such as the characteristics of the mating system and floral morphology influence this process will help in predicting which native species will experience the greatest impact of this potentially important process.

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Table 2.1A. Repeated measures ANOVA examining the response of visitation rate by all taxa to date, musk thistle presence, and number of common harebell flowers.

Variable	Effect	DF	F	p
	Musk thistle presence	1, 33	20.79	<0.001
Visits – All Taxa	Date	3, 33	1.45	0.25
	Common harebell flowers	1, 33	3.73	0.062

Table 2.1B. Repeated measures ANOVA examining the response of visitation rate by solitary bees to date and musk thistle presence.

Variable	Effect	DF	F	p
Visits – Solitary Bees	Musk thistle presence	1, 35	30.19	<0.001
	Date	3, 35	2.37	0.087

Table 2.1C. Repeated measures ANOVA examining the response of visitation rate by *Bombus* species to date, musk thistle presence, and the interaction between musk thistle presence and date.

Variable	Effect	DF	F	p
	Musk thistle presence	1, 32	2.61	0.12
Visits – <i>Bombus</i> species	Date	3, 32	4.29	0.012
	Musk thistle x Date	3, 32	3.73	0.048

Table 2.2. Repeated measures ANOVA examining the response of common harebell pollen grains per common harebell stigma to date, musk thistle presence, and the interaction between musk thistle presence and date.

Variable	Effect	DF	F	p
	Musk thistle presence	1, 56	0.10	0.75
Common harebell Pollen	Date	5, 56	8.81	<0.001
	Musk thistle x Date	5, 56	2.46	0.044

Table 2.3. Repeated measures ANOVA examining the response of common harebell flowers per plant to date, date by date, musk thistle presence, and the interaction between musk thistle presence and date.

Variable	Effect	DF	F	p
Common harebell flowers per plant	Musk thistle presence	1, 85	1.02	0.315
	Date	1, 85	5.44	0.022
	Date x Date	1, 85	6.03	0.016
	Musk thistle x Date	1, 85	6.25	0.014

Table 2.4. Repeated measures ANOVA examining the response of common harebell seeds per fruit to date, musk thistle presence, and the number of common harebell flowers.

Variable	Effect	DF	F	P
Seeds per Fruit	Musk thistle presence	1, 85	0.05	0.82
	Date	7, 85	5.57	<0.001
	Common harebell flowers	1, 85	8.40	0.005
	Mean <i>Bombus</i> visitation rate	1, 85	3.76	0.056

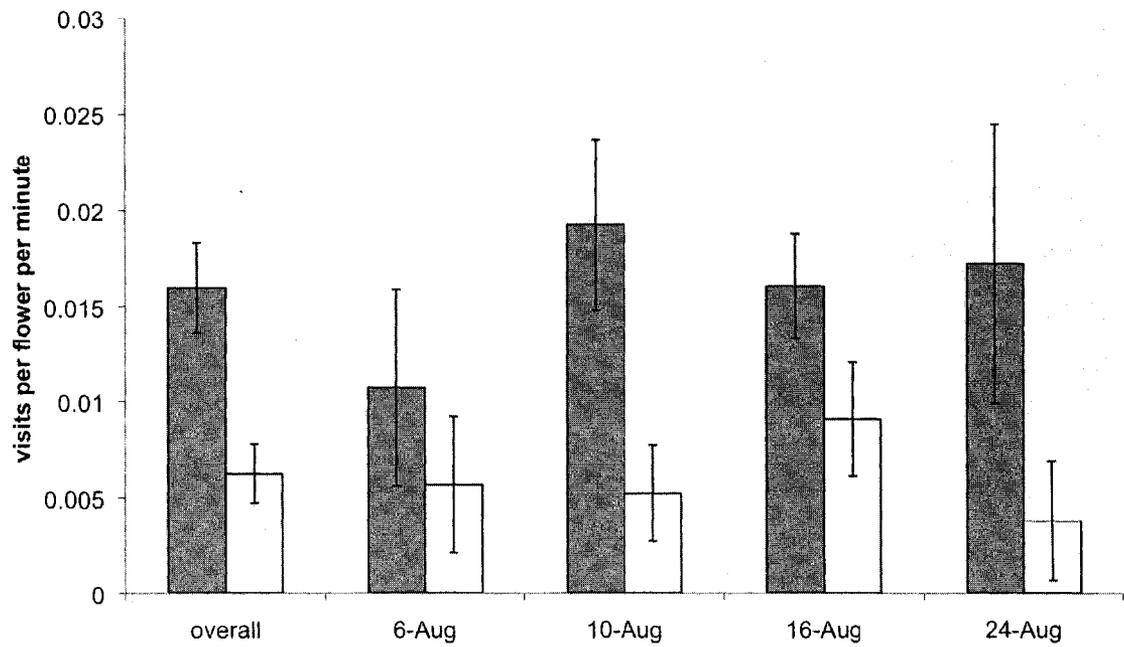


Figure 2.1A: Mean visitation rates for all taxa to common harebell per array with and without musk thistle. Solid bars indicate the absence of musk thistle while clear bars represent musk thistle presence. Means and  $\pm 1$  standard error of the mean for non-transformed data are presented.

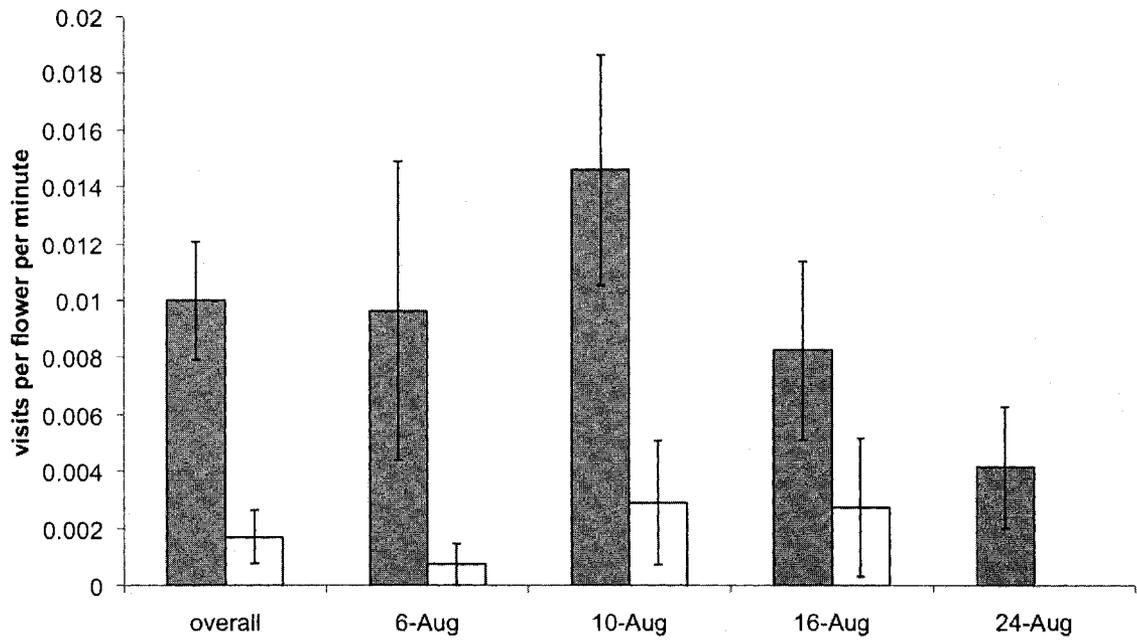


Figure 2.1B: Mean visitation rates for solitary bees to common harebell per array with and without musk thistle. Solid bars indicate the absence of musk thistle while clear bars represent musk thistle presence. Means and  $\pm 1$  standard error of the means for non-transformed data are presented.

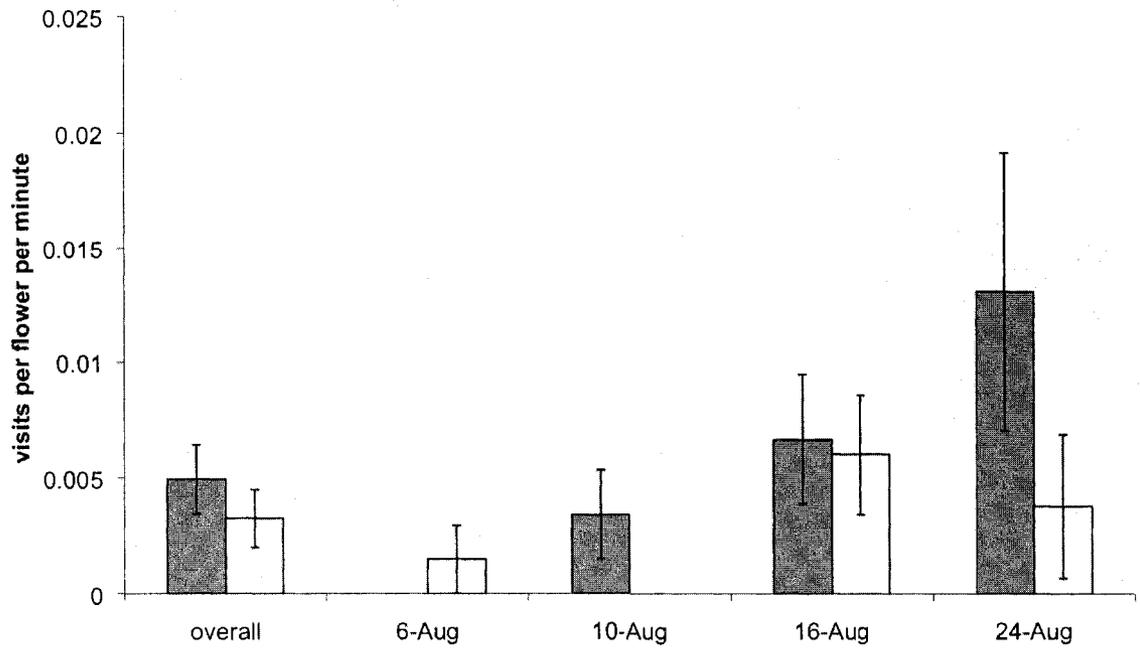


Figure 2.1C: Mean visitation rates for *Bombus* species to common harebell per array with and without musk thistle. Solid bars indicate the absence of musk thistle while clear bars represent musk thistle presence. Means and  $\pm 1$  standard error of the mean for non-transformed data are presented.

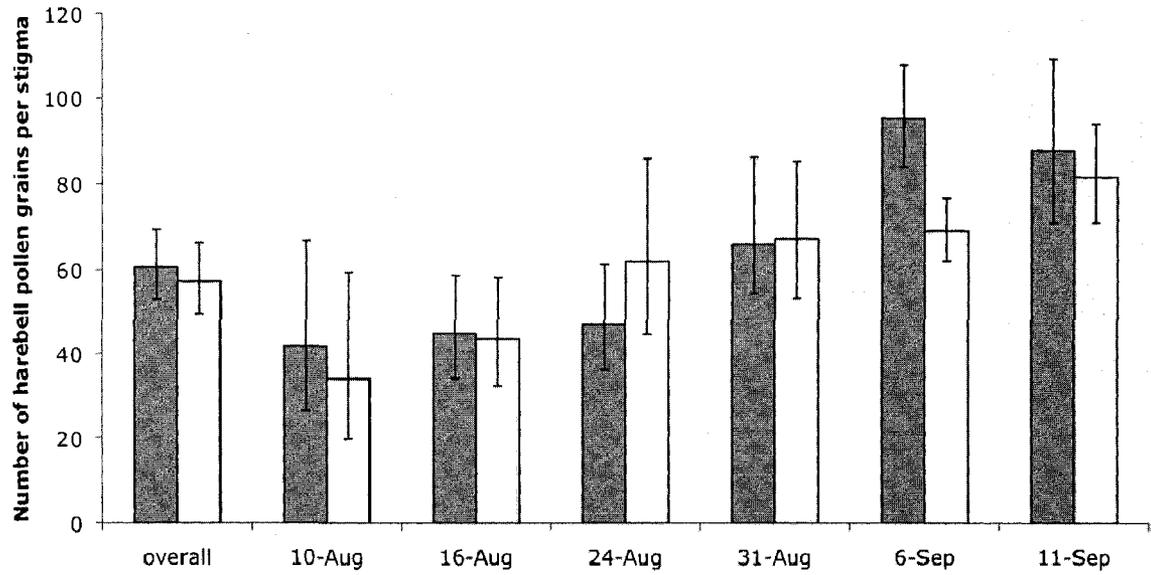


Figure 2.2: Mean number of common harebell pollen grains deposited on common harebell stigmas per array with and without musk thistle. Solid bars indicate the absence of musk thistle while clear bars represent musk thistle presence. Means and  $\pm 1$  standard error of the mean for non-transformed data are presented.

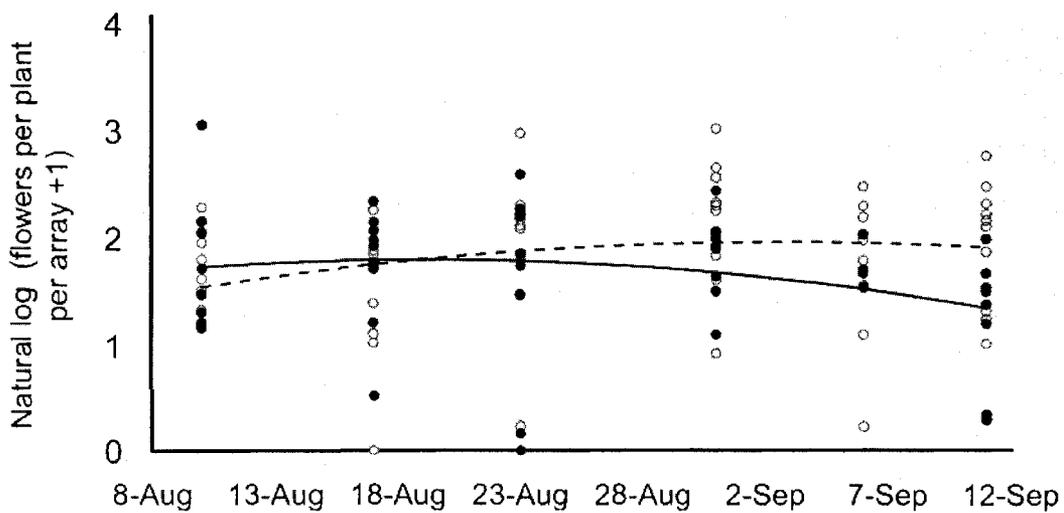


Figure 2.3: Mean number of flowers per plant with and without musk thistle for each array by date. Filled circles indicate the absence of musk thistle while clear circles represent musk thistle presence. The solid trend line represents arrays in the absence of musk thistle and the dashed line represents arrays with musk thistle presence.

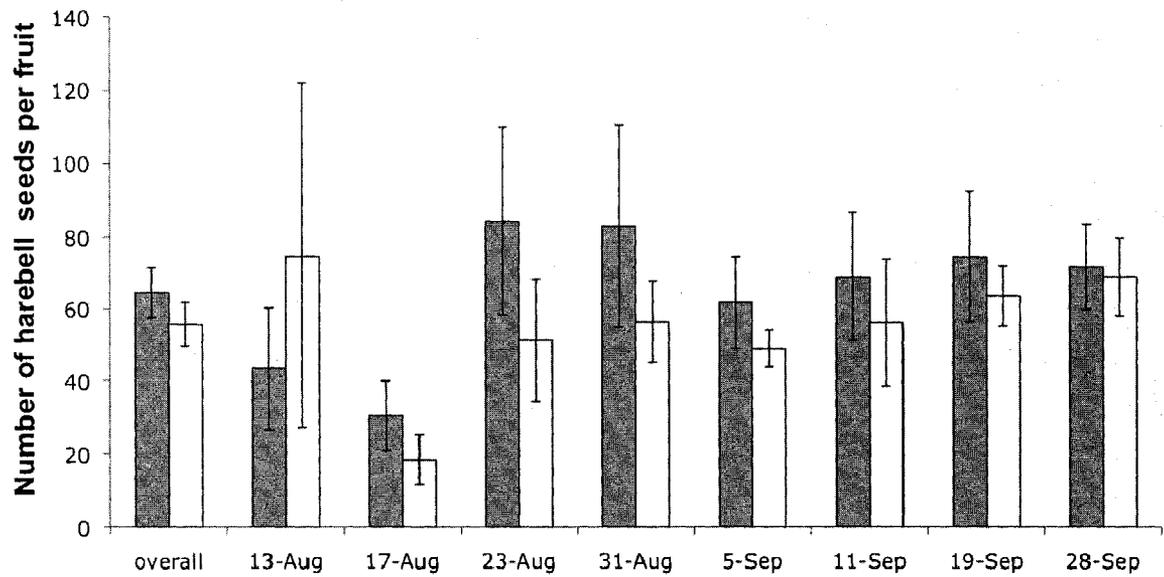


Figure 2.4: Mean number of common harebell seeds per fruit per array with and without musk thistle present. Solid bars indicate the absence of musk thistle while clear bars represent musk thistle presence. Means and  $\pm 1$  standard error the mean for non-transformed data are presented.

### CHAPTER THREE

DIRECT AND INDIRECT EFFECTS OF *MECINUS JANTHINUS* (COLEOPTERA)  
FEEDING ON THE EXOTIC PLANT, DALMATIAN TOADFLAX  
(SCROPHULARIACEAE), MEDIATED THROUGH FLOWER VISITORS

## Abstract

Herbivory and pollination are important determinants of female reproductive success in flowering plants. Plants must interact with herbivores and flower visitors simultaneously and interaction with one may alter the outcome of the interaction with the other. These indirect effects can have dramatic impacts on plant fitness. The purpose of this study was to examine whether the stem-boring weevil *Mecinus janthinus* (Germar, Curculionidae: Coleoptera) affects flower visitation rate and seed set of the exotic plant Dalmatian toadflax (*Linaria dalmatica* (L.) Mill. Scrophulariaceae). I compared the visitation rate, flower production, fruit production, and pollen limitation on Dalmatian toadflax plants with and without larval feeding by *M. janthinus*. Feeding by *M. janthinus* reduced the number of flowers and visitation rate, and there was a significant interaction between herbivory and flower number suggesting that the change in visitation rate was not solely a function of a reducing in flower abundance. Herbivory also had negative impacts on the reproductive success of Dalmatian toadflax. Total flower and fruit production decreased by over 30% in plants attacked by *M. janthinus*. However, plants with *M. janthinus* were not more pollen-limited than those without *M. janthinus*. This suggests that herbivory had primarily direct effects female reproductive success.

## Introduction

Herbivory and pollination are important determinants of female reproductive success in flowering plants. Herbivory often directly reduces resource availability and survivorship of plants and ultimately female reproductive success. Increases in visitation rate often directly increase pollen reception and subsequently seed set (Burd 1993, Ashman et al. 2004). Most research on plant-insect interactions has focused on these direct pair-wise relationships and has demonstrated that insects can have substantial direct effects on plant ecology and evolution (Bigger and Marvier 1998, Goodwillie et al. 2005). In addition, recent work has shown that plants simultaneously interact with multiple species and the interaction with one may affect the outcome of the relationship with another (Herrera et al. 2000, Morris et al. 2007). These indirect effects can take two forms. First, interaction chains (*sensu* Wootton 1993) result when one species changes the abundance of a second species with this change subsequently affecting a third species. Interaction chains are predictable as they are simply a series of direct effects and are discernable by measuring each pair-wise interaction in isolation (Wootton 1993). For example, herbivory may reduce the number of flowers per plant and this may lead to a concomitant decrease in number of flowers visited (Strauss et al. 1996, Lehtila and Strauss 1997). The second category of indirect effect, the interaction modification (*sensu* Wootton 1993), occurs when one species modifies the dynamics of the interaction between two other species. Interaction modifications are more difficult to predict, as they are not solely a function of changes in abundance and are often evident only after experimental manipulation (Wootton 1993, 1994). Most examples of interaction modifications in plant-pollinator-herbivore systems come from florivory studies (Strauss

and Irwin 2004, McCall and Irwin 2006). Florivory often results in changes in floral morphology or reward quality, and this may lead to a decrease in visitation rate independent of flower abundance (Johnson et al 1995, Irwin and Brody 1998, Mothershead and Marquis 2000). Vegetative herbivory is ubiquitous in flowering plants. However, relatively few studies that have examined the effect of vegetative herbivory on plant-pollinator interactions. Most of these studies have found that herbivory can also lead to changes in flower abundance as well as floral quality such as reward amount, flower size, and flower type and lead to decreases in visitation rate (Strauss et al. 1996, Mothershead and Marquis 2000, Hamback 2001). These results, coupled with the observations that seed set in many plants is pollen limited, suggests herbivory may lower female reproductive success by reducing resource availability and decreasing visitation rate.

The purpose of this study was to examine whether the exotic stem-boring weevil *Mecinus janthinus* (Germar, Curculionidae: Coleoptera) affects flower visitation and seed set of the exotic plant Dalmatian toadflax (*Linaria dalmatica* (L.) Mill. Scrophulariaceae). This system provides an interesting template to examine these interactions. Both Dalmatian toadflax and *M. janthinus* are native to Eurasia. Dalmatian toadflax is now invasive in North American and the biological control agent *M. janthinus* has been released to control Dalmatian and yellow toadflax populations. Many exotic plants are introduced without the full suite of enemies found in their native range and experience reduced herbivory in their invaded range This lack of enemies is an important component of the Enemy Release Hypothesis (Keane and Crawley 2002), and it underlies classical biological control programs. Furthermore, many exotic plants rely on flower

visitors for sexual reproduction and native and exotic flower visitors readily visit introduced plants (Memmott and Waser 2002, Lopezaraiza-Mikel et al. 2007).

I addressed three questions: 1) Does herbivory by *M. janthinus* directly alter flower number and floral traits of Dalmatian toadflax? 2) Does herbivory affect visitation rate through interaction chains or interaction modifications? 3) Does herbivory increase pollen limitation due to decrease in visitation rate? I predicted that herbivory would reduce flower number and lead to a decrease in the number of flowers visited. I also predicted that herbivory would alter floral morphology and/or quality leading to a decrease in visitation rate independent of the number of flowers. Finally, I expected that decreases in visitation rate would lead to greater pollen limitation in Dalmatian toadflax plants attacked by *M. janthinus* than in herbivore-free plants.

## **Methods**

### Study Species

Dalmatian toadflax is native to Mediterranean regions of Eurasia. It was introduced into the United States in the late 17<sup>th</sup> century as an ornamental and is now invasive in much of Canada and the western and northern United States (Alex 1962; Vujnovic and Wein 1997). It is a short-lived perennial that reproduces by both seed and rhizomes. Individual plants are made up of multiple stems that can each reach up to 1.5 meters in height and produce a simple raceme of yellow, zygomorphic flowers (Whitson et al. 2002). Each fruit can produce up to 300 seeds and individual Dalmatian toadflax plants can produce up to 500,000 seeds per growing season (Robocker 1974). Flowers are hermaphroditic, protandrous, and self-incompatible and rely on flower visitors for sexual reproduction (Docherty 1982, Vujnovic and Wein 1997). Corollas remain closed

throughout the life of the flower and flower visitors must either pry open corollas or insert their proboscis between the petals to collect nectar contained in a spur. In other flowering plants, nectar robbing can reduce floral attractiveness and reward amount leading to a decrease in visitation and reproductive success (McCall and Irwin 2006 for review). Short-tongued bees pierce and rob flowers of Dalmatian toadflax (*Cariveau pers obs.*) and its congener yellow toadflax (Newman and Thomson 2005, Burkle et al. 2007). However, I did not observe any signs of robbing at this study site (*Cariveau pers obs.*).

*Mecinus janthinus* is a univoltine stem-boring weevil that was intentionally released into North America in 1991 as biological control agent of Dalmatian and yellow toadflax (De Clerck-Floate and Harris 2002). Adults feed on the flowers and leaves, and in the late spring, females chew small holes and oviposit into the stem where the larvae develop. Larval feeding results in reduced photosynthetic rates, transpiration rates, and stomatal conductance (Peterson et al. 2005). Larvae pupate in the stems, overwinter as adults, and emerge in the late spring the following year. *Mecinus janthinus* is the most effective biological control agent for controlling populations of Dalmatian toadflax in North America (Sing et al. 2005).

### Experimental Design

At the Colorado State University campus in Fort Collins, Colorado (CO), I grew 120 Dalmatian toadflax plants from seed and transplanted them into 10-liter plastic pots. Approximately 4 months after germination, I placed 7 or 8 potted plants per cage into 16 cages covered with spunbonded polyester. This allowed for photosynthetic light to pass but was impenetrable to *M. janthinus*. I randomly assigned 8 cages as beetle-present and 8 cages as beetle-free. In May 2007, I released 10 *M. janthinus* adults per plant into the

beetle-present cages for a total of 70 or 80 beetles per cage. I used *M. janthinus* adults that I extracted from Dalmatian toadflax stems that were collected the previous fall. I left the adults in the cages for six weeks allowing them to feed, mate, and oviposit in the stems.

Six weeks after introducing *M. janthinus* (July 19), I took plants out of the cages and removed all adults. I transferred the plants to the Colorado State Forest Tree Nursery located at an elevation of 1560 meters in Fort Collins, Colorado. The nursery is comprised of tree rows, fallow fields, and open grass meadows that contain a mixture of native and exotic species. There were few naturally blooming flowers present in the area and there were no blooming Dalmatian toadflax populations within 1 km of the study site. I placed the plants in 10 rows of 12 plants using a completely randomized design, with each row and individual plants within each row separated by 3 meters.

*Question 1: Does M. janthinus herbivory alter the number of flowers and floral traits?* – Once a week from July 19 to September 6, I counted all open flowers on each Dalmatian toadflax plant to estimate the number of flowers open per day per plant. On Aug 2, I randomly chose 30 beetle-attacked and 30 beetle-free plants, and using calipers, I measured the size of three haphazardly chosen flowers per plant. I recorded corolla width, flower length, and spur length (Fig. 3.1). From these data, I calculated flower area for each individual flower as the product of corolla length by corolla width. For all measurements, data were averaged for the 3 flowers from each plant, keeping plant as the experimental unit.

*Question 2: Does M. janthinus herbivory affect visitation rate to Dalmatian toadflax?* – On August 7, 14, and 21, I observed each flowering plant for 10 minutes. For

consistency, I conducted all observations between 0800 and 1300. This was also the time of peak insect activity (Cariveau *per obs.*). I recorded a visit when any part of the flower visitor entered the flower corolla and touched the reproductive parts of the flower. I calculated visitation rate as the number of flowers visited per minute.

*Question 3: Does M. janthinus herbivory influence female reproductive success of Dalmatian toadflax?* – Once a week I counted all open flowers on each plant and collected and counted all ripe fruits. I then summed these values to obtain a total number of flowers and fruits produced per plant. In addition, once a week from July 22 through Aug 16, I haphazardly chose six flowers each from 30 randomly-chosen beetle-present and 30 randomly-chosen beetle-free plants and brushed dehiscing anthers collected from flowers of non-study plants onto the receptive stigma. If a plant had fewer than 6 flowers, I designated half of the flowers as hand pollinated and half as open pollinated. I used jewelry tags to mark the hand and open pollinated flowers. I collected the marked fruits before dehiscence and counted the number of seeds in each fruit.

#### Data Analysis

*Question 1: Does M. janthinus herbivory alter the number of flowers and floral traits?* –To assess whether *M. janthinus* feeding affected daily number of flowers produced by Dalmatian toadflax, I used repeated measures mixed model ANOVA. The response variable was the number of flowers open per plant for each date. Date, *M. janthinus* presence, and their interaction were fixed effects and plant was a random effect. To test differences in flower measurements between the two herbivory treatments, I used a t-test with the assumption of unequal variances.

*Question 2: Does M. janthinus herbivory affect visitation rate to Dalmatian toadflax?* – I used a repeated measures mixed model ANOVA with visitation rate as the response variable and plant as a random effect. Plants without blooming flowers were not included in the analyses. I examined three different models to identify whether herbivory indirectly affected visitation rate through an interaction chain or an interaction modification.

Visitation Model 1: To determine whether herbivory changed visitation rate, I designated date, *M. janthinus* presence, and their interaction as fixed effects. A significant effect of herbivory in this test would indicate that *M. janthinus* feeding, *in general*, indirectly affects visitation rate. Visitation Model 2: I then tested whether the indirect effect of *M. janthinus* was an interaction chain by removing herbivory as a fixed effect and replacing it with the number of flowers so that the fixed effects were flower number, date, and their interaction. A significant affect would provide evidence for an interaction chain of *M. janthinus* reducing flower abundance leading to a reduction in visitation rate. Visitation Model 3: Finally, I tested for an interaction modification by adding *M. janthinus* presence back into the model so that number of flowers, *M. janthinus* presence, date, and their interactions were fixed effects. A significant effect of the interaction between *M. janthinus* presence and number of flowers on visitation rate would indicate that visitation rate is not solely a function of flower abundance but of *M. janthinus* feeding as well. I used a model selection approach to determine the best model from the candidate visit models 1, 2, and 3 (Hobbs and Hilborn 2007). This technique compares the set candidate models based on their respective AIC values. The product of

the model is an Aikake weight for each model with values from 0 to 1. These values indicate the relative strength of evidence for each model given the data.

*Question 3: Does M. janthinus herbivory influence female reproductive success of Dalmatian toadflax?* – To examine whether *M. janthinus* herbivory affected total number of flowers and total number of fruits per plant, I used a t-test with the assumption of unequal variances. I also used a mixed model ANOVA to examine whether *M. janthinus* presence affected pollen limitation. The response variable was seeds per fruit while pollen addition, *M. janthinus* presence, and their interaction were fixed effects. I did not have enough power to adequately assess whether pollen limitation changed with date and therefore averaged seeds per fruit per plant for each of the four treatment categories.

I conducted all mixed model ANOVAs using the MIXED procedure in SAS (v9.2, SAS Institute 2008). I used the Kendall-Rogers adjustment for degrees of freedom in the mixed models. For all repeated measures analyses, I chose the variance-covariance structure that resulted in the lowest Akaike's Information Criterion (AIC) value (Littel et al. 1996). All t-tests were analyzed using JMP (v7.0.2 SAS Institute 2007).

## **Results**

*Mecinus janthinus* adults oviposited and larvae developed in the stems of Dalmatian toadflax. Plants in cages where *M. janthinus* were released on average had  $44.9 \pm 3.8$  (max = 150 min = 3) adults per plant. These numbers are within the range found in other field studies (De Clerck-Floate and Miller 2002, Norton *unpublished data*). Some *M. janthinus* did oviposit on '*M. janthinus* free plants', but the average was much lower ( $2.1 \pm 0.41$ , max = 14, min = 0).

*Question 1: Does M. janthinus herbivory alter the number of flowers and floral traits?* –Dalmatian toadflax plants produced fewer flowers per day when attacked by *M. janthinus* (mean =  $19.66 \pm 1.12$  vs.  $28.67 \pm 1.43$ ,  $F_{1, 120} = 15.69$ ;  $p < 0.001$ , Fig. 3.2, Table 3.1). The number of flowers in both treatments increased with date until the end of the season ( $F_{8, 775} = 77.04$ ;  $p < 0.001$ , Fig. 3.2, Table 3.1). There was a significant interaction between number of flowers and date ( $F_{8, 775} = 7.29$ ;  $p < 0.001$ , Fig. 3.2, Table 3.1), with the *M. janthinus* plants having more flowers in mid-August while differences between treatments were minimal at the beginning and end of the season (Fig 3.2). Using a Bonferroni adjustment for multiple comparisons, I found fewer flowers on *M. janthinus* attacked plants on July 19 ( $26.4 \pm 5.3$  vs.  $9.2 \pm 2.1$ ,  $t_{824} = 3.96$ ,  $p < 0.001$ ), August 18 ( $53.6 \pm 3.7$  vs.  $28.9 \pm 3.6$ ,  $t_{824} = 5.66$ ,  $p < 0.001$ ), and August 26 ( $43.9 \pm 3.9$  vs.  $68.0 \pm 5.8$ ,  $t_{824} = 5.51$ ,  $p < 0.001$ )

Floral traits did not differ between *M. janthinus* presence or absence in corolla length ( $25.94 \pm 0.77$  vs.  $24.46 \pm 1.2$ ,  $t_{24.02} = -1.04$ ;  $p = 0.31$ ), corolla width ( $8.60 \pm 0.37$  vs.  $9.1 \pm 0.48$ ,  $t_{28.5} = 0.828$ ;  $p = 0.41$ ), spur length ( $11.44 \pm 0.48$  vs.  $10.07 \pm 0.73$ ,  $t_{24.7} = -1.57$ ;  $p = 0.13$ ), or flower area ( $225.63 \pm 13.3$  vs.  $229.59 \pm 20.81$ ,  $t_{22.3} = 0.16$ ;  $p = 0.87$ ).

*Question 2: Does M. janthinus herbivory affect visitation rate to Dalmatian toadflax?* –*Bombus* species and *Apis mellifera* comprised the majority of visitors (40.3% and 34.1%, respectively). Diptera species made up 20.2% of flower visitors and 5.4% were solitary bees. Visitation rate was lower for Dalmatian toadflax plants attacked by *M. janthinus* ( $0.471 \pm 0.073$  vs.  $0.226 \pm 0.0373$ , Visit Model 1:  $F_{1, 114} = 12.83$ ;  $p = 0.005$ , Fig. 3.3, Table 3.2). For both treatments, visitation rate increased with date but this effect was not significant ( $F_{2, 132} = 1.04$ ;  $p = 0.36$ , Table 3.2). In Visit Model 2 (daily number of

flowers per plant instead of *M. janthinus* feeding), visitation rate increased with number of flowers ( $F_{1, 259} = 66.01$ ;  $p < 0.001$ , Table 3.3). Date was not significant ( $F_{2, 210} = 2.23$ ;  $p = 0.11$ , Table 3.3).

Visit Model 3 (herbivory, number of flowers, and their interaction) indicated that *M. janthinus* feeding altered the per flower attractiveness of plants to flower visitors. Plants without *M. janthinus* experienced a greater positive relationship between visitation rate and number of flowers ( $F_{1, 287} = 15.08$ ;  $p < 0.001$ , Fig 3.3, Table 3.4). In this model, number of flowers was significant ( $F_{1, 245} = 54.73$ ;  $p < 0.001$ , Table 3.4) while *M. janthinus* feeding by itself and date were not (*M. janthinus* feeding:  $F_{1, 202} = 3.33$ ;  $p = 0.069$ ; Date:  $F_{2, 209} = 2.16$ ;  $p = 0.12$ , Table 3.4). Model selection indicated that the model with *M. janthinus* treatment, number of flowers, and their interaction was overwhelming the best model. (Aikake weights: *M. janthinus* feeding only:  $<0.01$ , Flower number only:  $<0.01$ ; *M. janthinus* feeding x number of flowers:  $>0.99$ )

*Question 3: Does M. janthinus herbivory influence female reproductive success of Dalmatian toadflax? – Mecinus janthinus* free plants produced 33% more flowers and 38% more fruits than those attacked by *M. janthinus* (Flowers:  $239.17 \pm 14.17$  vs.  $160.70 \pm 14.19$ ,  $t_{118} = -3.92$ ;  $p < 0.001$ , Fig 3.5A; Fruits:  $331.05 \pm 24.89$  vs.  $205.37 \pm 18.72$ ,  $t_{109.6} = -4.04$ ;  $p < 0.001$ ; Fig. 3.5B). Plants with *M. janthinus* had fewer seeds per fruit than those without *M. janthinus* but this was not significant ( $105.46 \pm 7.08$  vs.  $86.96 \pm 5.71$ ,  $F_{1,56} = 1.81$ ;  $p = 0.18$ , Fig 3.6, Table 3.5). Hand pollination increased seeds per fruit by 12.2% when averaged over both herbivory treatments ( $102.42 \pm 6.25$  vs.  $89.96 \pm 6.69$ ,  $F_{1,56} = 6.02$ ;  $p = 0.017$ , Fig. 3.6, Table 3.5). I did not find evidence of an interaction between *M. janthinus* herbivory and pollen addition ( $F_{1,56} = 0.011$ ;  $p = 0.74$ ; Fig. 3.6,

Table 3.5). However, hand-pollination of *M. janthinus* attacked plants significantly increased the number of seeds per fruit by 15.1% (82.6 vs. 97.3 least squared means;  $t_{56} = 1.97$ ,  $p = 0.054$ , Fig. 3.6). Hand pollination also increased seeds per fruit in *M. janthinus* free plants (99.86 vs. 111.07 least squared means) however this difference was not significant ( $t_{56} = 1.50$ ,  $p = 0.139$ ).

## Discussion

The findings of this study demonstrate that larval feeding by the classical biological control agent, *Mecinus janthinus*, has direct and indirect effects on Dalmatian toadflax. *Mecinus janthinus* feeding directly reduced the number of flowers produced per plant, and a number of other studies have found similar results (Strauss et al. 1996, Lehtila and Strauss 1997, Hamback 2001). Larval feeding by *M. janthinus* reduces photosynthetic rates, transpiration rates, and stomatal conductance of Dalmatian toadflax, and this likely leads to a decrease in the amount of resources available for flower production (Peterson et al. 2005). As demonstrated by Visit Model 2, this decrease in the number of flowers resulted in concomitant decreases in visitation rate. This was expected as flower visitors often prefer plants with larger inflorescences (Hamback 2001, Mitchell et al. 2004, Naug and Arathi 2007). While flower abundance by itself was an important factor affecting visitation rate, Visit Model 3 revealed a significant effect of an interaction between *M. janthinus* feeding and flower number on visitation rate. This suggests the presence of an interaction modification, as the change in flower visitor behavior was not solely a function of flower abundance. Furthermore, model selection analysis indicated that the model with this interaction modification best describes this indirect effect. These results suggest that, in addition to reducing flower abundance, *M. janthinus* feeding altered one

or more floral traits that are also important determinants of visitation rate. In some systems, vegetative herbivory alters the size and morphology of individual flowers and this leads to a decrease in visitation (Strauss et al 1996, Mothershead and Marquis 2000). However, I found no evidence for an effect of *M. janthinus* feeding on individual Dalmatian toadflax flower measurements. Herbivory may also alter other floral characteristics important to visitation such as pollen production, nectar amount, and nectar quality (Strauss et al 1996, Strauss et al 2001, Adler et al. 2006). While *M. janthinus* feeding may have affected these floral traits, I did not measure these attributes in this study.

Feeding by *M. janthinus* also affected female reproductive success of Dalmatian toadflax. Larval feeding reduced the total seasonal number of flowers and fruits per plant by 33% and 38%, suggesting a direct effect of herbivory on female reproductive success. In addition, pollen addition increased seeds per fruit by 15% in *M. janthinus* attacked plants however the effect was similar to plants without *M. janthinus*. Other studies have found similar results. For example, Hamback (2001) found that spittlebug (Cercopidae: Hemiptera) feeding reduced the number of flowers and number of flowers visited in *Rudbeckia hirta* (Asteraceae) while female reproductive success was primarily influenced directly by herbivory. In contrast, Mothershead and Marquis (2000) used hand clipping to demonstrate that leaf herbivory reduced seed set of *Oenothera macrocarpa* (Onagraceae) by reducing corolla size and visitation rate. Each species of herbivore can have a distinct set of impacts on a plant, and this may influence the magnitude of direct and indirect effects through flower visitors. For example, Peterson et al. (2002) found that while *M. janthinus* had significant negative impacts on a number of metabolic pathways of

Dalmatian toadflax, the leaf-feeding herbivore, *Calophasia lunula* (Noctuidae: Lepidoptera) had negligible effects on these processes. Even within a species of herbivore, various life stages may have differing effects on plant floral traits and female fitness. For example, *M. janthinus* adults also feed on leaves and flowers, and a number of studies have demonstrated that florivory reduces plant attractiveness to floral visitors (see McCall and Irwin 2006 for review). I examined only larval herbivory in this study and expect that adults would increase the magnitude of direct and indirect effects. Therefore, to assess the total effect of feeding by *M. janthinus* both adults and larvae must be considered. In addition to effects on visitation rate and female reproductive success, herbivory may have other important plant-level impacts through flower visitors. I measured only correlates of female fitness (i.e. seeds per fruit) and other work has demonstrated that herbivory may also affect male fitness such as by reducing the amount of pollen produced (Strauss et al. 1996). Fewer insects visited plants with *M. janthinus* and this likely decreased the quantity of pollen exported. A decrease in the overall pollen pool may also lead to an increase in pollen limitation (Campbell and Motten 1985).

Recent studies have demonstrated that biological control agents can have negative non-target effects on native plants and communities, revealing the potential risks of this management technique (e.g. Louda et al. 1997, Pearson and Callaway 2003). Ideally, biological control agents should have large impacts on the target plant to maximize the benefit to cost ratio of release (Pearson and Callaway 2003, Sing et al. 2005). My findings of a >30% reduction in flowers and fruits and additional indirect effects of herbivory mediated through flower visitors support many other studies showing that larval feeding by *M. janthinus* has considerable negative effects on Dalmatian toadflax

(Sing et al. 2005, Peterson et al. 2005). These results coupled with Breiter and Seastedt's (2007) finding that *M. janthinus* is highly host-specific in both greenhouse and field settings suggest that *M. janthinus* is likely an efficacious and safe management tool for controlling Dalmatian toadflax populations. Even if biological control agents are highly host specific, they may still impose negative non-target effects on native communities. For example, Pearson and Callaway (2003) found that the seed-feeding biological control agent, *Urophora affinis* (Tephritidae: Diptera) persists in large numbers on *Centaurea maculosa* (Asteraceae) seed heads and provides a food subsidy to deer mice. This dramatically increases deer mice population size and potentially alters food web dynamics. However, non-target effects of biological control are not always negative. In particular, herbivores may ameliorate the impacts of exotic plants independent of their influence on exotic plant populations. For example, recent work has demonstrated that exotic plants may affect native plants through competition for pollination services (e.g. Chittka and Shurkens 1999, Brown et al. 2002, Cariveau and Norton *in press*). Exotic plants often have larger floral displays and rewards and these plants may draw away flower visitors from native plants. *Mecinus janthinus* feeding reduced flower number and visitation rate to Dalmatian toadflax and this may translate into a reduction in the ability of the plant to compete with native plants for pollination services. A more complete understanding of the negative and positive impacts of biological control on both the target exotic plants and native communities will greatly aid in successful management of invasive plants.

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Table 3.1: Repeated measures ANOVA examining the response daily flower number per plant to date and *Mecinus janthinus* presence.

Variable	Effect	DF	F	p
	<i>Mecinus janthinus</i>	1, 120	15.69	< 0.001
Flowers Per Day	Date	8, 775	77.04	< 0.001
	<i>M. janthinus</i> x Date	8, 775	7.29	< 0.001

Table 3.2. Visit Model 1: Repeated measures ANOVA examining the response of visitation rate to date and *Mecinus janthinus* presence.

Variable	Effect	DF	F	p
Visitation Rate	<i>Mecinus janthinus</i>	1, 114	12.83	0.005
	Date	2, 132	1.04	0.36

Table 3.3. Visit Model 2: Repeated measures ANOVA examining the response of visitation rate to number of flowers and date and *Mecinus janthinus* presence.

Variable	Effect	DF	F	p
Visitation Rate	Number of flowers	1, 259	66.01	< 0.001
	Date	1, 210	2.23	0.111

Table 3.4. Visit Model 3: Repeated measures ANOVA examining the response of visitation rate to number of flowers, date and *Mecinus janthinus* presence.

Variable	Effect	DF	F	p
Visitation Rate	<i>Mecinus janthinus</i>	1, 202	3.33	0.069
	Number of flowers	1, 245	54.73	< 0.001
	<i>M. janthinus</i> x flowers	1, 287	15.08	< 0.001
	Date	2, 209	2.16	0.118

Table 3.5. Mixed-model ANOVA examining the response of seeds per fruit to *Mecinus janthinus* presence and number of flowers per plant.

Variable	Effect	DF	F	p
	<i>Mecinus janthinus</i>	1, 56	1.81	0.184
Seeds per Fruit per Plant	Pollination treatment	1, 56	6.02	0.017
	Pollination x <i>M. janthinus</i>	1, 56	0.11	0.74

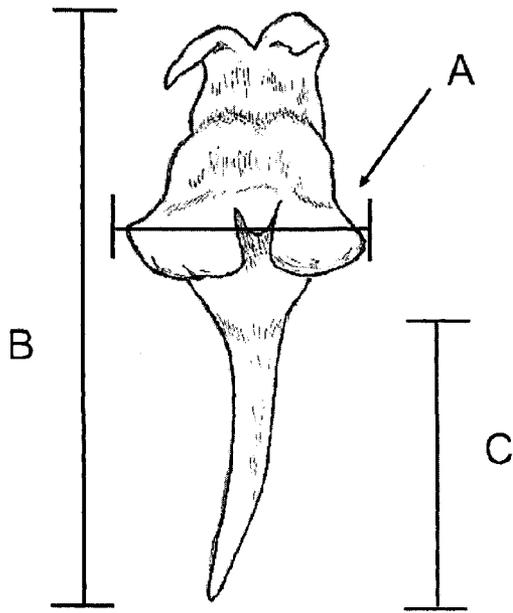


Figure 3.1. Frontal view of an individual Dalmatian toadflax flower. Measurements included A) Corolla width, B) Flower Length and C) Spur length. Illustration by Janet Hardin.

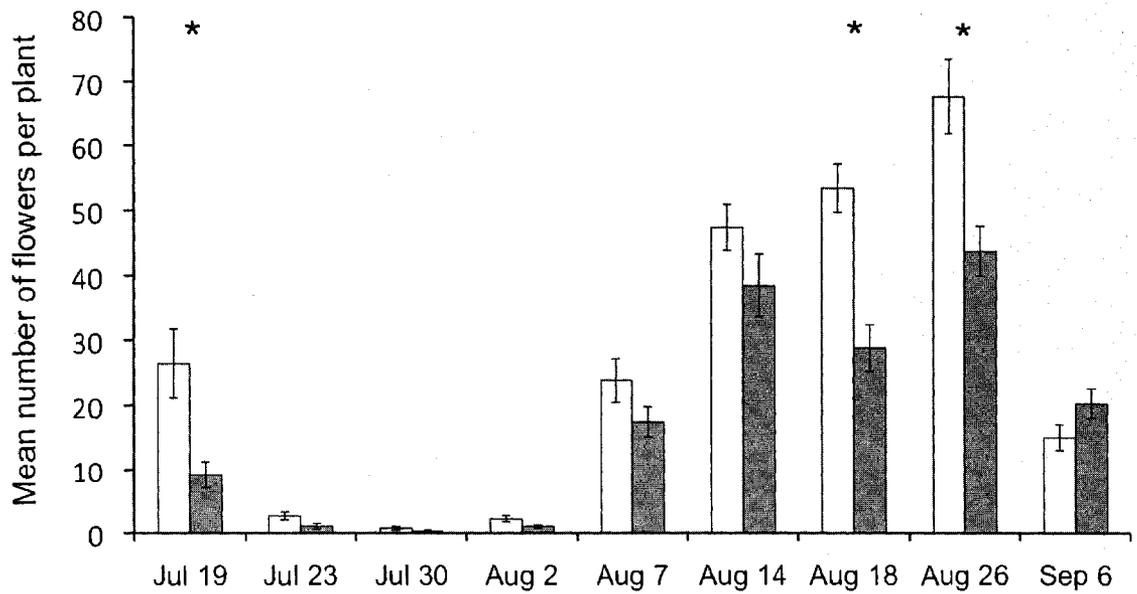


Figure 3.2. Number of Dalmatian toadflax flowers per day with and without *Mecinus janthinus* herbivory. Clear bars represent plants without *M. janthinus* feeding. Filled bars represent plants attacked by *M. janthinus*. Error bars represent  $\pm 1$  standard error. \* indicate  $p < 0.001$  and are adjusted using the Bonferroni test for multiple comparisons.

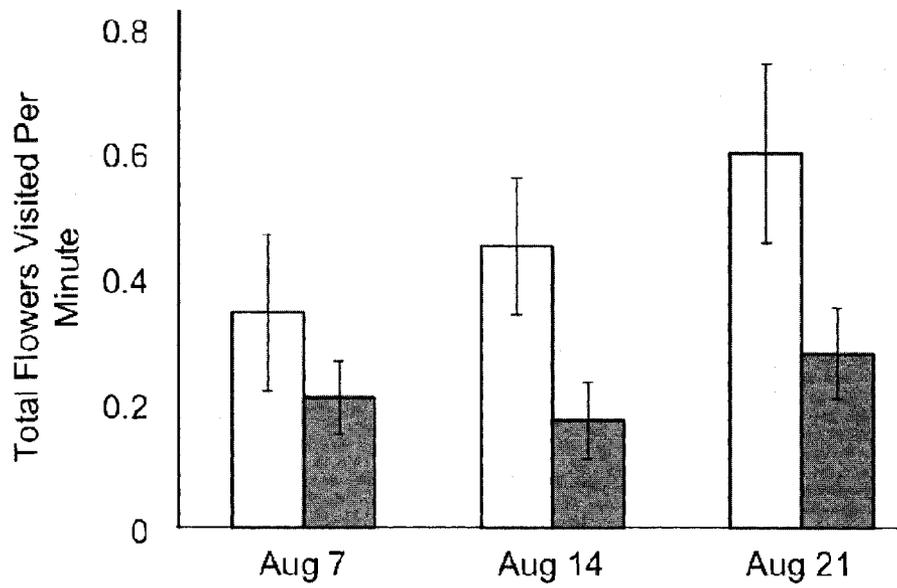


Figure 3.3. The mean number flowers visited per minute per plant. Clear bars represent plants without *Mecinus janthinus* feeding. Filled bars represent plants attacked by *M. janthinus*. Error bars represent  $\pm 1$  standard error.

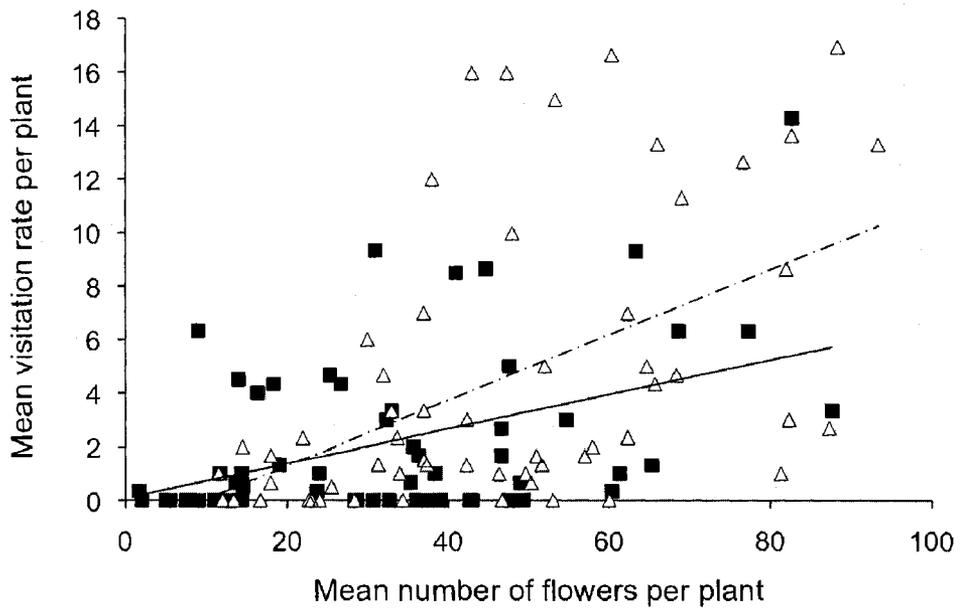


Figure 3.4. The mean visitation rate and flower number for each plant. Clear triangles and dashed line represent plants without *Mecinu janthinus* feeding. Filled squares and solid line represent plants attacked by *M. janthinus*.

Figure 3.5A

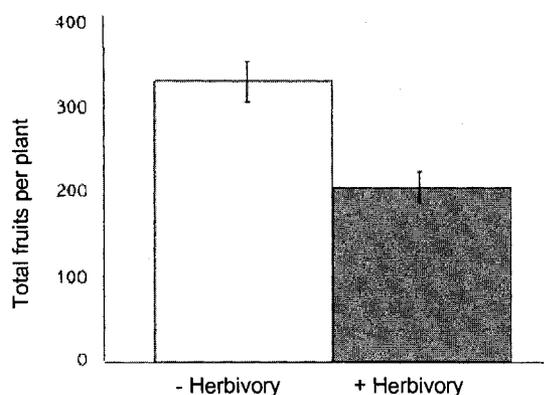


Figure 3.5B

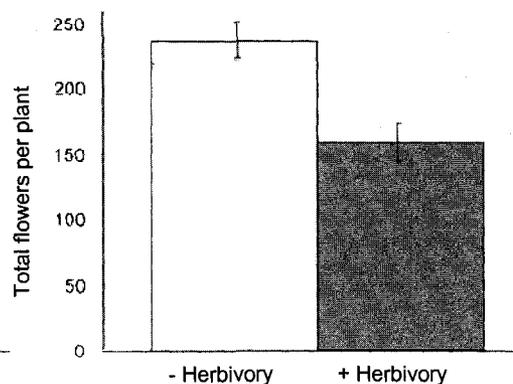


Figure 3.5B. The mean of the total number of flowers produced over the entire season. Clear bars represent plants without *Mecinus janthinus* feeding. Filled bars represent plants attacked by *M. janthinus*. Error bars represent  $\pm 1$  standard error.

Figure 3.5A. The mean of the total number of fruits produced over the entire season. Clear bars represent plants without *Mecinus janthinus* feeding. Filled bars represent plants attacked by *M. janthinus*. Error bars represent  $\pm 1$  standard error.

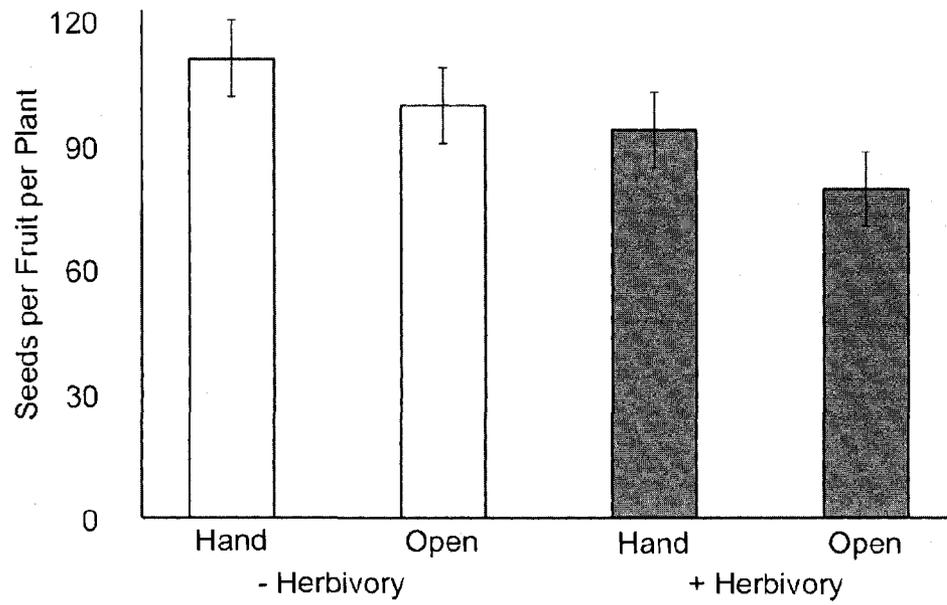


Figure 3.6. Least squares means of seeds per fruit. Clear bars represent plants without *Mecinus janthinus* feeding. Filled bars represent plants attacked by *M. janthinus*. Error bars represent  $\pm 1$  standard error.