



# Spatially contingent interactions between an exotic and native plant mediated through flower visitors

Daniel P. Cariveau and Andrew P. Norton

D. P. Cariveau ([cariveau@lamar.colostate.edu](mailto:cariveau@lamar.colostate.edu)) and A. P. Norton, Dept of Bioagricultural Sciences and Pest Management, Graduate Degree Program in Ecology, Colorado State Univ., Fort Collins, CO 80523, USA.

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 we 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? We 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, we 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*. We 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*. Our results suggest interactions between plant species through flower visitors may depend on spatial scale.

Many plant species interact through shared mutualists and this can lead to fitness and population-level consequences (reviewed by 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 (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, Laverty 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 Laverty 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; reviewed by Bjerknes et al. 2007).

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 the amount of available rewards (Klinkhamer et al. 2001). Thus in close proximity, interspecific floral switching may occur frequently and 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. To our knowledge no studies have examined how spatial proximity of native and exotic plants may influence interactions through flower visitors.

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

We collected *Carduus nutans* rosettes in the early spring of 2005 and grew them in 10 l pots on the Colorado State University campus. We 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, we removed all open and thus potentially pollinated flowers prior to the experimental manipulations.

### Small-plot experiments

This experiment took place 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, we constructed 20 2.5 × 2 m plots each containing an array of potted plants. We 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.

We 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 represented in this study represent the lower range of population sizes for each species. Within plots, each plant was separated by 1 m. We 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 (Moeller and Geber 2005). Our 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 (Chittka and Shurkens 2001, Totland et al. 2006, Lopezaraiza-Mikel

et al. 2007). We 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 we conducted 20-min observations of the native plants. We observed half of the flowers in a plot in one 10-min bout and the other half in a second 10-min bout. For each observation bout, we 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, we pooled data from the two observation bouts. We also recorded the type of flower visitor as *Bombus* species, solitary bee, Lepidoptera, Diptera, or other. As flower number can affect visitation rate, we also counted the number of *M. fistulosa* flower and *C. nutans* inflorescences.

We observed plots once every five to seven days during a 5-week period (5 Aug.–31 Aug.). Due to low flower numbers in some plots we 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 unpubl.).

**Interplant movements.** Once every 5 to 7 days from 16 to 29 August, we conducted 20-min 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 we recorded which species of plant the flower visitor departed and which species it visited next.

### **Stigma pollen counts**

Once a week from the 15 to 29 of August, we collected up to 10 stigmas from each blooming *M. fistulosa* plant. For plants with fewer than 100 flowers, we collected only 10% of the stigmas to minimize the effect of stigma collection on overall seed set. We 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). We 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 27 August through 26 October, we collected all fruits from *M. fistulosa* as they ripened and counted all seeds within each fruit. For each collection date, we 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. We then averaged the number of seeds per fruit for each plant to obtain a plot average for each collection date.

### **Spatial interactions**

We examined the effect of distance from *C. nutans* patches on visitation rates to *M. fistulosa* from 12 through 21 July

2006. This experiment was conducted at the Colorado State Forest Tree Nursery located at an elevation of 1560 m in Fort Collins, Colorado. The nursery is comprised of tree rows, fallow fields, and open grass meadows that contains a mixture of native and exotic plants.

We constructed eight plots in which we placed two potted *M. fistulosa* plants each at 0, 1, 5 and 15 m from a center point in which we added or removed *C. nutans*. We 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, we 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 our plots.

At each experimental plot we 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 unpubl.). We 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 we either removed or added *C. nutans* and allowed the flower visitors to acclimate for 24 h 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*. We 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 we 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, we randomly chose the order of the distances at which to observe the plants. We used this same order for the second set of observations. Observation bouts lasted between 15 and 40 min, 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**

We used SAS ver. 9.1 to conduct all statistical analyses. 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, we used a mixed model ANOVA in PROC MIXED. We used the arcsine (square root (x)) transformation for the visitation rate response variables. We 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. We 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, we 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. We 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 we removed them from the final model.

We used a repeated measures design in all of these models to accommodate the multiple measurements on separate dates. We 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. We 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 we 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. We determined the expected probability by calculating the proportion of inflorescences available for each plant species. We then compared the total observed with the total expected switches using a  $\chi^2$  test (PROC FREQ).

## Results

### Small-plot study

#### Visitation rates

We observed a total of 297 flower visitors during 26 h 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 *Monarda 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, Table 1). Visitation rate decreased with date ( $F_{4,54} = 24.77$ ,  $p < 0.001$ ; Fig. 1, Table 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.

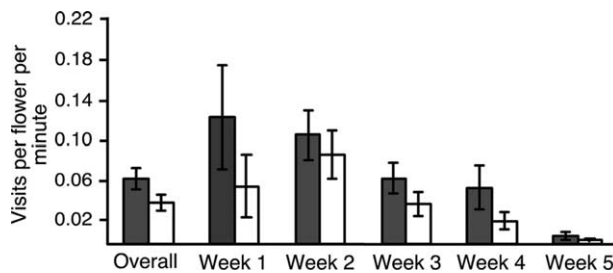


Figure 1. Visits per *M. fistulosa* flowers per minute by date. Solid bars represent visitation rate without *C. nutans*. Open bars indicate the *C. nutans* treatment. Analysis was conducted using the arcsine (square root) transformation. Data presented are non-transformed means. Error bars represent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

#### Interplant movement

Forty flower visitors made 106 interplant switches in 280 min 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 we expected this to occur 14 times ( $\chi^2 = 4.22$ ,  $DF = 1$ ,  $p = 0.04$ ; Fig. 2). Similarly, flower visitors leaving *C. nutans* moved to another *C. nutans* plant 49 times; we expected approximately 28 switches ( $\chi^2 = 25.50$ ,  $DF = 1$ ,  $p < 0.001$ ; Fig. 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. 3a, Table 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. 3a, Table 2). Date was not significant ( $F_{1,30} = 0.93$ ,  $p = 0.41$ ; Table 2) yet the interaction between date and treatment was significant ( $F_{2,30} = 3.53$ ,  $p = 0.042$ ; Table 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 we 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. 3b, Table 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 with date ( $F_{1,33} = 16.07$ ,  $p < 0.001$ ;

Table 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	0.36

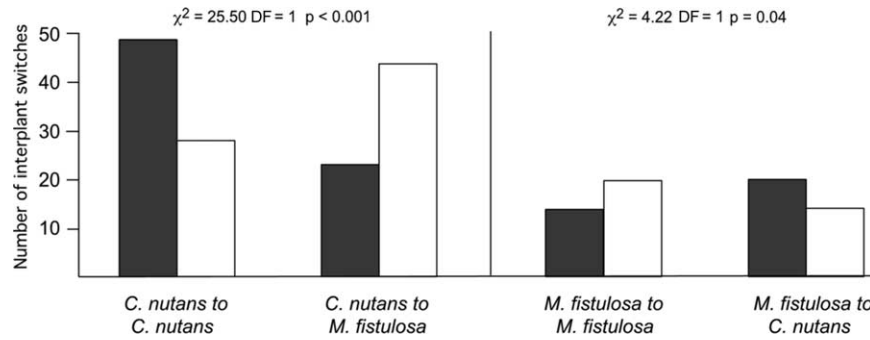


Figure 2. Observed and expected movements between plant species by flower visitors in the presence of *C. nutans*. Solid bars indicate the observed number of switches. Open bars represent the expected number of switches.

Fig. 3b, Table 3), but the interaction between date and treatment was not significant and we removed this from the final model. We 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 4) although this effect was not significant. Seeds per fruit decreased as date increased ( $F_{7,66} = 2.70$ ,  $p = 0.016$ ; Table 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 4). The

interaction between date and treatment and the number of *C. nutans* were not significant and we 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. 4, Table 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. 4, Table 5) however, as this was our main hypothesis in this experiment, we continued to investigate differences at each distance for the treatment effect. *Monarda fistulosa* flowers at a distance of 0 and 15 m 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. 4) while *M. fistulosa* flowers at 1 and 5 m 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. 4). Visitation rate decreased as date increased ( $F_{5,46} = 2.63$ ,  $p = 0.036$ ; Table 4). Distance in the absence of its interaction with treatment was not significant in the model ( $F_{3,46} = 0.46$ ,  $p = 0.71$ ; Table 4). 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 we removed these covariates from the final analysis.

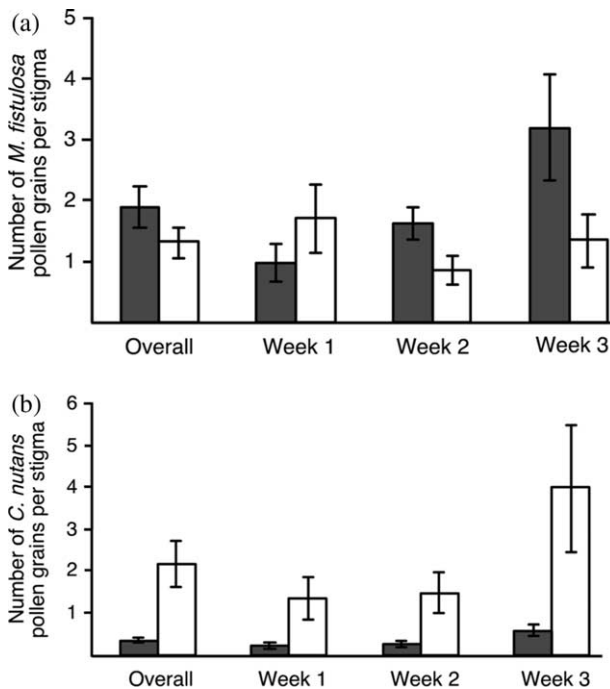


Figure 3. (a) Mean number of *M. fistulosa* pollen grains per stigma without and with the presence of *C. nutans*. Solid bars represent number of *M. fistulosa* pollen grains per stigma without *C. nutans*. Open bars indicate the *C. nutans* treatment. Analysis was conducted using the  $\ln(x+1)$  transformation. (b) Mean number of *C. nutans* pollen grains per stigma without and with the presence of *C. nutans*. Solid bars represent number of *C. nutans* pollen grains per stigma without *C. nutans*. Open bars indicate the *C. nutans* treatment. Analysis was conducted using the  $\ln(x+1)$  transformation. Data presented are non-transformed means. Error bars represent  $\pm 1$  SE.

### Discussion

Plants may compete through flower visitors by decreasing visitation rate, transferring heterospecific pollen grains, or a combination of both processes. We found evidence that the presence of an invasive plant influences flower visitor behavior in relation to a native plant. Most importantly, we 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

Table 2. Repeated measures ANOVA examining the response of *Monarda fistulosa* pollen grains per *M. fistulosa* stigma to *M. fistulosa* flower number, date, *C. nutans* presence and the interaction between *C. nutans* presence and date.

Variable	Effect	DF	F	p
<i>M. fistulosa</i> pollen	Date	2, 30	0.93	0.41
	<i>C. nutans</i> presence	1, 30	7.16	0.012
	<i>C. nutans</i> × date	2, 30	3.53	0.042
	<i>Monarda fistulosa</i> flower	1, 30	19.43	<0.001

Table 3. Repeated measures ANOVA examining the response of *Carduus nutans* pollen grains per *M. fistulosa* stigma to date, *C. nutans* presence and their interaction.

Variable	Effect	DF	F	p
<i>C. nutans</i> pollen	Date	2, 31	12.30	<0.001
	<i>C. nutans</i> presence	1, 31	16.07	<0.001

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 (Campbell and Motten 1985, Brown et al. 2002) and our results add to the growing knowledge of how native and invasive plants interact through flower visitors (Bjerknes et al. 2007). Furthermore, our study provides evidence that interspecific competition among plants may occur at spatial scales much greater than the zone of rhizosphere overlap.

We suspect that we 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. Our data suggest that competition is operating on a larger spatial scale (1–5 m) than facilitation (<1 m). 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 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 our 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*) we were unable to collect these data.

*Monarda fistulosa* seed set was 31% greater in the absence of *C. nutans* in our 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 we found decreases in visitation to *M. fistulosa* in the presence of *C. nutans* at 1 and 5 m. We suspect that these large differences would result in changes in seed set; however, we 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 our 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 our small plot study, we found some support for this pattern as plots with more *M. fistulosa* flowers had more conspecific pollen grains and seeds per fruit. However, we found no effect of *M. fistulosa* flower number on visitation rate. Natural populations of *M. fistulosa* vary in size and the numbers we used in our experiments are at the low range of what occurs in natural settings (Cariveau unpubl.) 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 our 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

Table 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 *C. 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

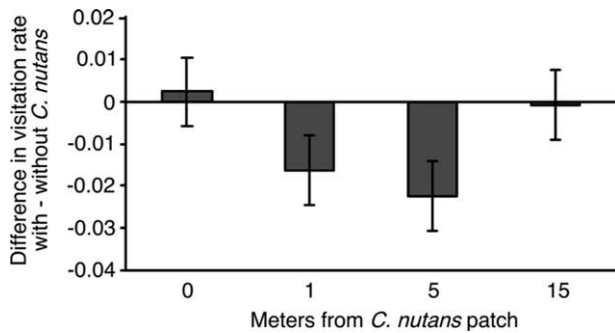


Figure 4. Difference in visitation rate at each distance point. Negative values indicate lower visitation rates in the presence of *Carduus nutans*. Analysis was conducted using the arcsine (square root) transformation. Data presented are non-transformed means. Error bars represent  $\pm 1$  SE.

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 (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 unpubl.). 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

Table 5. Repeated measures ANOVA examining the response of *Monarda fistulosa* visitation rate to date, distance from *Carduus nutans* patch, presence of *C. nutans* and the interaction between distance and *C. 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	0.71
	Presence $\times$ distance	3, 46	2.32	0.105

that could result in negative impacts to native plants. Our 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.

**Acknowledgements** – A. Brody, C. Brown, A. Cariveau, D. Vázquez, B. Kondratieff, J. Hardin and D. Naug greatly improved this manuscript with their helpful comments. We thank J. Hardin and T. Marty for assistance in fieldwork and B. Balderstone for spending numerous hours counting pollen grains.

## References

- Andres, L. A. and Goeden, R. D. 1971. The biological control of weeds by introduced enemies. – In: Huffaker, C. B. (ed.), Biological control. Proc. AAAS Symp. on biological control. Plenum Press, pp. 143–162.
- Ashman, T. L. et al. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. – *Ecology* 85: 2408–2421.
- Bartomeus, I. et al. 2008. Contrasting effects of invasive plants in plant–pollinator networks. – *Oecologia* 155: 761–770.
- Beattie, A. J. 1971. Technique for study of insect-borne pollen. – *Pan. Pac. Entomol.* 47: 82.
- Bjerknes, A. L. et al. 2007. Do alien plants invasions really affect pollination success in native plant species? – *Biol. Conserv.* 138: 1–12.
- Brown, B. J. et al. 2002. Competition for pollination between an invasive species (purple loosestrife) and a native congener. – *Ecology* 83: 2328–2336.
- Burd, M. 1994. Bateman principle and plant reproduction – the role of pollen limitation in fruit and seed set. – *Bot. Rev.* 60: 83–139.
- Cambell, D. R. and Motten, A. F. 1985. The mechanisms of competition for pollination between between two forest herbs. – *Ecology* 66: 554–563.
- Chittka, L. and Schurkens, S. 2001. Successful invasion of a floral market – an exotic Asian plant has moved in on Europe's riverbanks by bribing pollinators. – *Nature* 411: 653.
- Cruden, R. W. et al. 1984. The pollination biology and breeding system of *Monarda fistulosa* (Labiatae). – *Oecologia* 64: 104–110.
- Feldman, T. S. et al. 2004. When can two plant species facilitate each other's pollination? – *Oikos* 105: 197–207.
- Ghazoul, J. 2002. Flowers at the front line of invasion? – *Ecol. Entomol.* 27: 638–640.
- Ghazoul, J. 2004. Alien abduction: disruption of native plant–pollinator interactions by invasive species. – *Biotropica* 36: 156–164.
- Ghazoul, J. 2006. Floral diversity and the facilitation of pollination. – *J. Ecol.* 94: 295–304.
- Grabas, G. P. and Laverty, T. M. 1999. The effect of purple loosestrife (*Lythrum salicaria* L.; Lythraceae) on the pollination and reproductive success of sympatric co-flowering wetland plants. – *Ecoscience* 6: 230–242.
- Jones, K. N. 2004. Do dandelion flowers influence seed set of a native plant (*Delphinium nuttallianum*) in subalpine meadows? – *Am. Midl. Nat.* 151: 201–205.
- Klinkhamer, P. G. L. et al. 2001. Small-scale spatial patterns determine ecological relationships: an experimental example using nectar production rates. – *Ecol. Lett.* 4: 559–567.

- Knight, T. M. 2003. Floral density, pollen limitation, and reproductive success in *Trillium grandiflorum*. – *Oecologia* 137: 557–563.
- Larson, B. M. H. and Barrett, S. C. H. 2000. A comparative analysis of pollen limitation in flowering plants. – *Biol. J. Linn. Soc.* 69: 503–520.
- Laverly, T. M. 1992. Plant interactions for pollinator visits – a test of the magnet species effect. – *Oecologia* 89: 502–508.
- Lehtila, K. and Strauss, S. Y. 1997. Leaf damage by herbivores affects attractiveness to pollinator in wild radish, *Raphanus raphanistrum*. – *Oecologia* 111: 396–403.
- Levine, J. M. et al. 2003. Mechanisms underlying the impacts of exotic plant invasions. – *Proc. R. Soc. Lond. B* 270: 775–781.
- Loperaraiza-Mikel, M. E. et al. 2007. The impact of an alien plant on a native plant–pollinator network: an experimental approach. – *Ecol. Lett.* 10: 539–550.
- Memmott, J. and Waser, N. M. 2002. Integration of alien plants into a native flower–pollinator visitation web. – *Proc. R. Soc. Lond. B* 269: 2395–2399.
- Mitchell, C. E. et al. 2006. Biotic interactions and plant invasions. – *Ecol. Lett.* 9: 726–740.
- Moeller, D. A. 2004. Facilitative interactions among plants via shared pollinators. – *Ecology* 85: 3289–3301.
- Moeller, D. A. and Geber, M. A. 2005. Ecological context of the evolution of self-pollination in *Clarkia xantiana*: population size, plant communities, and reproductive assurance. – *Evolution* 59: 786–799.
- Moragues, E. and Traveset, A. 2005. Effect of *Carpobrotus* spp. on the pollination success of native plant species of the Balearic Islands. – *Biol. Conserv.* 122: 611–619.
- Murphy, S. D. and Aarsen, L. W. 1995. Reduced seed set in *Elytrigia repens* caused by allelopathic pollen from *Phleum pratense*. – *Can. J. Bot.* 72: 1417–1422.
- Rathcke, B. 1983. Competition and facilitation among plants for pollination. – In: Real, L. (ed.), *Pollination biology*. Academic Press, pp. 305–329.
- Rathcke, B. 1988. Interactions for pollination among coflowering shrubs. – *Ecology* 69: 446–457.
- Strauss, S. Y. et al. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. – *Am. Nat.* 147: 1098–1107.
- Totland, O. et al. 2006. Effects of an exotic plant and habitat disturbance on pollinator visitation and reproduction in a boreal forest herb. – *Am. J. Bot.* 93: 868–873.
- Traveset, A. and Richardson, D. M. 2006. Biological invasions as disruptors of plant reproductive mutualisms. – *Trends Ecol. Evol.* 21: 208–216.
- Waser, N. M. and Fugate, M. L. 1986. Pollen precedence and stigma closure – a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. – *Oecologia* 70: 573–577.
- Williams, N. M. et al. 2001. Variation in native bee faunas and its implications for detecting community change. – *Conserv. Ecol.* 5: 57–89.