

Increased relative abundance of an invasive competitor for pollination, *Lythrum salicaria*, reduces seed number in *Mimulus ringens*

Rebecca J. Flanagan · Randall J. Mitchell ·
Jeffrey D. Karron

Received: 14 December 2009 / Accepted: 8 June 2010 / Published online: 29 June 2010
© Springer-Verlag 2010

Abstract When exotic plant species share pollinators with native species, competition for pollination may lower the reproductive success of natives by reducing the frequency and/or quality of visits they receive. Exotic species often become numerically dominant in plant communities, and the relative abundance of these potential competitors for pollination may be an important determinant of their effects on the pollination and reproductive success of co-occurring native species. Our study experimentally tests whether the presence and abundance of an invasive exotic, *Lythrum salicaria* L. (Lythraceae), influences reproductive success of a co-flowering native species, *Mimulus ringens* L. (Phrymaceae). We also examine the mechanisms of competition for pollination and how they may be altered by changes in competitor abundance. We found that the presence of *Lythrum salicaria* lowered mean seed number in *Mimulus ringens* fruits. This effect was most pronounced when the invasive competitor was highly abundant, decreasing the number of seeds per fruit by 40% in 2006

and 33% in 2007. Reductions in the number of seeds per fruit were likely due to reduced visit quality resulting from *Mimulus* pollen loss when bees foraged on neighboring *Lythrum* plants. This study suggests that visit quality to natives may be influenced by the presence and abundance of invasive flowering plants.

Keywords Invasive species · *Lythrum salicaria* · *Mimulus ringens* · Pollen loss · Pollinator

Introduction

When exotic species invade flowering plant communities, competition for pollination may lower the reproductive success of co-occurring native species (Chittka and Schurkens 2001; Ghazoul 2004; Morales and Aizen 2006; Bjerknes et al. 2007). These competitive effects generally occur through two nonexclusive mechanisms (Waser 1978). First, pollinator preference for an invasive species may reduce the frequency of pollinator probes to native plants, lowering conspecific pollen deposition (Waser 1983; Campbell 1985; Brown et al. 2002; Kandori et al. 2009). Second, invasive species may lower the quality of pollinator probes to native plants by interfering with conspecific pollen transfer. For example, pollen of native species may be deposited onto stigmas of the competitor, lowering seed set of the native species (Feinsinger and Tiebout 1991; Bell et al. 2005; Murcia and Feinsinger 1996; Larson et al. 2006; Morales and Traveset 2008). Reduced conspecific pollen deposition may also result from the loss of native species' pollen when bees groom while foraging on an invasive species (Flanagan et al. 2009; Mitchell et al. 2009a).

The direction of interactions for pollination and the magnitude of competitive effects on pollination success of

Communicated by Steven Johnson.

R. J. Flanagan · J. D. Karron
Department of Biological Sciences,
University of Wisconsin-Milwaukee,
P.O. Box 413, Milwaukee, WI 53201, USA

Present Address:
R. J. Flanagan (✉)
Department of Biology, Indiana University,
Bloomington, IN 47405, USA
e-mail: rjflana@gmail.com

R. J. Mitchell
Department of Biology, University of Akron,
Akron, OH 44325-3908, USA

native species may depend on both the presence and relative abundance of invasive competitors (Bjerknes et al. 2007; Munoz and Cavieres 2008). Increases in the abundance of an invasive species may change the direction of interactions for pollination from facilitation to competition, or may increase the severity of competition that already occurs at equal species abundances (Munoz and Cavieres 2008; Morales and Traveset 2009). Earlier studies of competition for pollination often examined equal abundances of two competing species native to the same region, simulating sympatry of naturally co-occurring species and effectively comparing presence versus absence of a competitor (Waser 1978, 1983; Campbell 1985; Galen and Gregory 1989). More recent studies have demonstrated that competitive effects may vary with relative abundance, generally becoming more intense with increasing competitor abundance (Caruso 2002; Ghazoul 2006; Munoz and Cavieres 2008; Takakura et al. 2009; Morales and Traveset 2009). However, the effect of one species on another might be context-dependent, changing from positive to neutral to negative with changes in relative abundance (Rathcke 1983; Mitchell et al. 2009a). Invasive species commonly develop extensive and dense populations, and the relative and absolute abundances of these invasives are likely to influence the magnitude of competitive effects on natives (Bjerknes et al. 2007). For example, the presence of an invasive may increase local patch size, attracting more pollinators and potentially increasing pollinator visitation to natives (Rathcke 1983; Feldman et al. 2004; Totland et al. 2006; Munoz and Cavieres 2008; Molina-Montenegro et al. 2008). However, highly abundant invasive plants may monopolize pollinator visits—despite increases in local pollinator attraction—reducing the frequency of probes to less abundant native species (Rathcke 1983; Munoz and Cavieres 2008). Highly abundant invasive plants may also diminish visit quality to natives through increases in interspecific pollen transfer (Bjerknes et al. 2007; Morales and Traveset 2008). In particular, it has been hypothesized that an increase in the number of competitor flowers probed during interspecific pollinator transitions should exacerbate pollen losses when invasives are abundant (Morales and Traveset 2008). Reductions in visit quality may occur whether or not the frequency of pollinator probes to natives is affected (Waser 1983; Larson et al. 2006; Lopezaraiza-Mikel et al. 2007).

This study experimentally tests whether the presence and abundance of an invasive exotic, *Lythrum salicaria* L. (Lythraceae), influences reproductive success of a co-flowering native species, *Mimulus ringens* L. (Phrymaceae). In an earlier study, we found that bumblebee workers moving between *Mimulus* and *Lythrum* flowers deposited significantly less conspecific pollen onto *Mimulus* stigmas compared to foragers that only visited *Mimulus* (Flanagan

et al. 2009). In the present study, we varied *Lythrum* abundance in competition with a constant number of *Mimulus* plants in order to address the following questions: (1) does the presence and abundance of *Lythrum* influence the quantity and quality of pollinator probes to *Mimulus*, and (2) does the presence and abundance of *Lythrum* influence *Mimulus* reproductive success?

Materials and methods

Study species

Mimulus ringens L. (Phrymaceae) is a wetland perennial herb native to central and eastern North America (Grant 1924). Populations tend to be small, often fewer than 50 individuals. At our field site in southeastern Wisconsin, USA, *Mimulus* flowers from mid-July through mid-September. The large blue zygomorphic flowers open at 5 a.m. and last for half a day (Mitchell et al. 2004). Daily floral display size ranges from 1 to 22 flowers (Karron et al. 2009).

Flowers are pollinated by bumblebee workers as they contact the stigma and anthers with their tongues and faces (Karron et al. 1995a, b; Mitchell et al. 2004). A single *Bombus* probe typically deposits 3,000–7,000 pollen grains (Flanagan et al. 2009) onto the bilobed stigma. Nearly all flowers produce seed capsules (Karron et al. 2004; Mitchell et al. 2005). Each flower has approximately 6,000 ovules, and the number of seeds per fruit following a single probe is typically 1,600–2,300 seeds (Karron et al. 2006). Seed number for flowers receiving three probes is significantly (43.6%) higher than seeds per fruit for flowers receiving a single probe, suggesting that stigmas often fail to receive a saturating load of pollen during an initial probe (Karron et al. 2006).

Mimulus ringens flowers are self-compatible and have a mixed-mating system (Karron et al. 1995a). Outcrossing rates vary widely within and among populations, and are influenced by population density (Karron et al. 1995a, b), floral display size (Karron et al. 2004), floral morphology (Karron et al. 1997), and the presence of competitors for pollination (Bell et al. 2005). Karron et al. (2009) also documented striking variation in selfing rate among adjacent flowers which reflects increased deposition of geitonogamous (among-flower, within-display) self pollen as bumble bees probe consecutive flowers on a floral display.

Lythrum salicaria L. (Lythraceae) is a wetland perennial herb native to Eurasia and invasive in North America (Hager and McCoy 1998; Blossey et al. 2001; Farnsworth and Ellis 2001). In the species' invasive range, it frequently grows in large, dense stands (Thompson et al. 1987; Mal et al. 1992; Farnsworth and Ellis 2001). Individual *Lythrum*

plants may have up to 50 spikes of densely packed, showy magenta flowers that are very attractive to pollinators (Mal et al. 1992). *Lythrum salicaria* is tristylous, producing three floral morphs that differ in the relative positions of stigmas and anthers (Darwin 1877; Mal et al. 1992; Agren 1996). *Lythrum* flowers from July through October, overlapping substantially with the phenology of *Mimulus*. The introduced range of *Lythrum* overlaps broadly with the range of *Mimulus* and they occasionally co-occur. In areas of sympatry, bumblebee pollinators move freely between the two species.

Experimental arrays

To address the influence of *Lythrum* presence and abundance on *Mimulus* reproductive success, we constructed two-dimensional arrays using potted plants of each species. These plants were grown in 35.6-cm diameter pots at the University of Wisconsin-Milwaukee Field Station (Saukville, WI, USA). Competition treatments were established by placing pots of each species into a square grid with 1-m spacing between adjacent pot centers. This design enabled us to sequentially expose all competition treatments to the same local pollinator assemblage. Plants not used in an array were stored in a pollinator-free screenhouse.

In each array, we positioned 15 *Mimulus* plants in a checkerboard arrangement (Fig. 1). In the control treatment, no *Lythrum* plants were present (Fig. 1a). To quantify interactions for pollination when *Mimulus* and *Lythrum* were at an equal relative abundance, we added 15 *Lythrum* plants to the checkerboard design (Fig. 1b). To quantify interactions for pollination when *Lythrum* was at a high relative abundance, we established a second competition treatment consisting of 15 *Mimulus* plants and 41 *Lythrum* plants by surrounding 30 plants configured as in the equal abundance treatment with a ring of additional *Lythrum*

plants (Fig. 1c). This design keeps the spacing of *Mimulus* plants constant but simulates a small patch of *Mimulus* embedded in a larger matrix of *Lythrum*. All three *Lythrum* style morphs were represented equally in each competitive array. We trimmed each vigorously-growing *Mimulus* plant to eight flowers to reflect the size of a typical *Mimulus* floral display in nature (Karron et al. 2009). These eight flowers were spread evenly across the plant, mimicking displays on unmanipulated plants. We trimmed the *Mimulus* displays at 5 a.m., before pollinators became active. We did not manipulate *Lythrum* displays, which already reflected display sizes typical of nearby populations (Flanagan et al. 2009).

We established each competition treatment or control on a separate day. A set of two different competition treatments and one control comprised a block. Because we established all three treatments in the same location, we allowed 1 day between observations so that pollinators could acclimate to each newly-established array. We used a fresh set of *Mimulus* plants for each block. At the end of each block, we returned *Mimulus* plants to the screenhouse to protect them from further pollinator visitation and pests while fruits ripened. Therefore, except for a brief window when *Mimulus* plants were in the experimental array, all the plants used in this experiment were subject to very similar environmental conditions. The use of potted plants also gave us a high degree of control over the nutrient and moisture status of plants used in the experiment, allowing us to minimize variation in plant resource status. On the day after each array was established, we quantified pollinator visitation and then tagged flowers to measure seeds per fruit. Each block lasted 6–8 days, depending on weather conditions. Pollinator visitation rates and the resulting seeds per *Mimulus* fruit were measured for two blocks during August 2006 and three blocks during August 2007.

Pollinator visitation patterns

On each observation day, we identified pollinators to species and recorded pollinator visitation rates during 10-min intervals scheduled every 30 min between 5 a.m. (prior to the arrival of the first bee) and 1 p.m. (after all *Mimulus* stigmas had closed). Because multiple bees frequently foraged simultaneously in the plot, we divided the central 30 positions in each array (e.g., 15 *Mimulus* plants and, if present, 15 *Lythrum* plants) into five sections, each with three *Mimulus* and three (or zero) *Lythrum* plants. During each 10-min window, we recorded the number of probes to *Mimulus* flowers and *Lythrum* flowers on plants in one section. This allowed us to record all bee probes to the six (or three) plants during the observation period. We were able to observe and record all probes using this method, even when multiple bees were present. We rotated among the

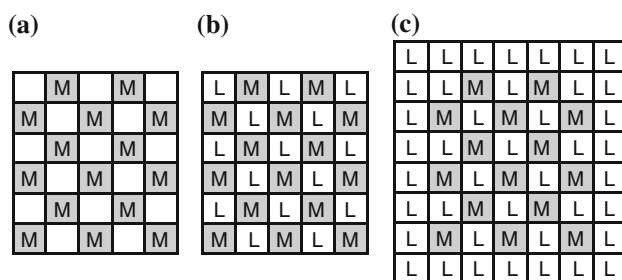


Fig. 1 Diagrammatic representation of experimental design used to quantify interactions for pollination. **a** Monospecific arrays of *Mimulus* were compared to arrays where **b** *Mimulus* and *Lythrum* were at an equal relative abundance and **c** where *Lythrum* outnumbered *Mimulus* by approximately 3:1. *M* *Mimulus*, *L* *Lythrum*. Plot sizes for **a** and **b** were 5 m × 5 m; for **c** 7 m × 7 m. There is 1 m spacing between adjacent plant centers in all plots

five sections randomly throughout the day. In the high *Lythrum* abundance treatments, we collected visitation rate data for the inner 30 plants only. We used these data to estimate probes per *Mimulus* flower per hour over the 8-h window of observation.

For all analyses, we used JMP version 5.1.2 (SAS Institute 2004). To test the effects of *Lythrum* presence and abundance on the rate of visitation to *Mimulus*, we used ANOVA (general linear models procedure) with a single measurement of probes per flower per hour for each 8-h observation day as the unit of replication ($n = 15$). In the model, competition treatment and year were fixed effects, and block nested within year was a random effect. We treated year as a fixed factor because 2 years are not sufficient to allow a confident estimate of levels of variation among all possible years (Newman et al. 1997). To characterize among-year variation in the composition of pollinator species probing *Mimulus* or *Lythrum* during the 10-min visitation intervals, we used G tests of independence.

To determine whether *Lythrum* presence and abundance influenced pollinator arrivals to arrays, in 2007 we censused the number of bumblebees foraging on *Mimulus* and *Lythrum* plants in the arrays. We counted all bees visiting plants of either species in the entire array once every 30 min from 5 a.m. until 1 p.m. We then tested for treatment and block effects on the mean number of bees visiting each array using a two-way ANOVA.

Patterns of pollinator foraging between species

In mixed-species experimental arrays, we quantified two additional aspects of pollinator foraging behavior: the extent to which bees discriminated among species, preferentially visiting one species over another, and the extent to which bees moved between species in a single foraging bout. Immediately following each visitation rate census, we recorded the complete floral visitation sequence for each pollinator foraging in an array during a 20-min period. We noted the species and location of each plant visited. To test for pollinator preference, we compared the frequency of visits to plants of each species in the array to expected values which were calculated based on the availability of plants of each species. We tested for significant departure from these expected values with a G test (Sokal and Rohlf 1995; Husband and Barrett 1992). In this analysis, we used blocks as a replicated measure. Therefore, we present a G statistic for the pooled frequencies across blocks of each treatment (G_P) and for heterogeneity among blocks of each treatment (G_H).

We also used G tests to evaluate the tendency of foragers to make heterospecific (*Mimulus*-to-*Lythrum* or *Lythrum*-to-*Mimulus*) versus conspecific (*Mimulus*-to-*Mimulus* or *Lythrum*-to-*Lythrum*) transitions in mixed species arrays. In

this analysis, we used blocks as a replicated measure, and expected values for plant-to-plant transitions were calculated based on the frequency of visits pollinators made to each species (Ippolito et al. 2004). Therefore, deviation from expected values should be independent from patterns of pollinator preference (Husband and Barrett 1992; Dafni et al. 2005).

Pollinator foraging data were also used to quantify the number of *Lythrum* flowers probed consecutively during each *Mimulus*-to-*Lythrum*-to-*Mimulus* transition in mixed species arrays. We used ANOVA (general linear models procedure) to test the effects of competition treatment on the number of *Lythrum* flowers probed per interspecific (*Mimulus*-to-*Lythrum*-to-*Mimulus*) transition. In the model, competition treatment and year were both fixed effects. The random effect of block nested within year was not significant (see “Results”) and so block effects were pooled in the final model. In this analysis, individual *Mimulus*-to-*Lythrum*-to-*Mimulus* pollinator transitions ($n = 120$) served as the unit of replication.

Number of seeds per *Mimulus* fruit

To quantify the effects of competition treatment on *Mimulus* reproductive success, we tied labeled plastic tags to the pedicels of two randomly-selected flowers on all 15 *Mimulus* plants in each array. We tagged a total of 450 flowers (2 flowers \times 15 plants \times 3 competition treatments \times 5 blocks). We harvested the fruits upon ripening (~ 30 days) and counted all seeds in each fruit using a dissecting microscope.

We used ANOVA (general linear models procedure) to test the effects of *Lythrum* presence and abundance on the number of seeds per *Mimulus* fruit. In the model, competition treatment and year were both fixed effects. If our data are analyzed with year as a random effect, the only change is that the differences among years become insignificant. The random effect of block nested within year was not significant (see “Results”) and so block effects were pooled in the final model. In this analysis, a single measurement of mean seeds for each treatment day was the unit of replication ($n = 15$).

We also used ANCOVA to examine the influence of increasing *Lythrum* abundance on seeds per *Mimulus* fruit. We scored competition treatment as a numerical covariate, corresponding to the relative abundance of *Lythrum* to *Mimulus*, and tested for its effects on *Mimulus* seeds per fruit. When *Lythrum* was absent, we scored *Lythrum* abundance as 0; when *Lythrum* and *Mimulus* plants were equally abundant, we scored *Lythrum* abundance as 1; and when *Lythrum* was nearly three times as abundant as *Mimulus*, we scored *Lythrum* abundance as 3. This model also incorporated the categorical variable year and the interaction of

Lythrum abundance and year. As in the ANOVA described above, non-significant block effects were pooled and mean seed number per treatment day was the unit of replication. The non-significant interaction term was also dropped from the model in the final analysis.

Results

Variation in pollinator species composition

Bombus impatiens was the most prevalent visitor to both *Mimulus* and *Lythrum* flowers in 2006, accounting for 90% of 294 probes to *Mimulus* and 99% of 3,294 probes to *Lythrum*. The remaining 10% of probes to *Mimulus* and 1% of probes to *Lythrum* were by *Bombus vagans*. In 2007, *Bombus impatiens* was less prevalent, accounting for 46% of 292 probes to *Mimulus* and 63% of 1,923 probes to *Lythrum*. *Bombus vagans* also visited frequently during 2007, accounting for 54% of probes to *Mimulus* and 32% of probes to *Lythrum*. Two other species, *B. griseocollis* and *Apis mellifera*, also made occasional visits to *Lythrum* during 2007. Overall, the composition of *Bombus* species visiting *Mimulus* differed significantly between 2006 and 2007 ($G = 146.50$, $df = 1$, $P < 0.001$). Similarly, the composition of *Bombus* species visiting *Lythrum* differed significantly between years ($G = 1,339.66$, $df = 2$, $P < 0.001$).

In 2007, the presence and abundance of *Lythrum* significantly influenced the composition of pollinator species visiting arrays ($G = 232.67$, $df = 2$, $P < 0.001$). When only *Mimulus* was present, *B. impatiens* accounted for 61% of pollinator species visiting arrays and *B. vagans* accounted for 39%. When *Mimulus* and *Lythrum* were equally abundant, *B. impatiens* accounted for 45% of pollinator species visiting arrays, and *B. vagans* accounted for 54%. When *Lythrum* was at a high relative abundance, *B. impatiens* accounted for 80% of pollinator species visiting arrays, and *B. vagans* accounted for 20%.

Pollinator visitation rates and patterns of pollinator arrivals to arrays

There was a marginally non-significant effect of competition treatment on the rate of pollinator visitation to *Mimulus* ($F_{2,6} = 4.83$, $P = 0.06$). In 2006, when no *Lythrum* was present, *Mimulus* received 3.77 ± 0.47 (mean ± 1 SE) visits per flower per hour. When *Lythrum* was present at an equal relative abundance, *Mimulus* received 1.72 ± 0.47 visits per flower per hour. When *Lythrum* was at a high relative abundance, *Mimulus* received 1.92 ± 0.47 visits per flower per hour. In 2007, when no *Lythrum* was present, *Mimulus* received 1.64 ± 0.38 visits per

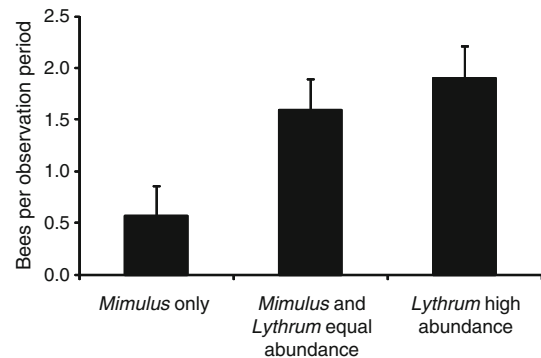


Fig. 2 Effect of presence and relative abundance of *Lythrum* on the mean number of pollinators visiting arrays ($+1$ SE, $n = 65$) in 2007

flower per hour. When *Lythrum* was present at an equal relative abundance, *Mimulus* received 1.05 ± 0.38 visits per flower per hour. When *Lythrum* was at a high relative abundance, *Mimulus* received 1.83 ± 0.38 visits per flower per hour. The rate of pollinator visitation per *Mimulus* flower did not vary significantly as a function of year, but did vary significantly among blocks ($F_{3,6} = 5.08$, $P = 0.04$).

The total number of bees visiting arrays varied significantly among competition treatments (one-way ANOVA, $F_{2,59} = 5.42$, $P = 0.007$). In 2007, we observed three times as many bees in arrays with equal frequencies of *Mimulus* and *Lythrum* as were present in arrays without *Lythrum* plants (Fig. 2). When *Lythrum* was at a high relative abundance, nearly four times as many bees visited the arrays. In an a posteriori test, pollinator arrivals to arrays were significantly lower in the absence of *Lythrum*, but did not differ significantly between the two *Lythrum* abundances (Tukey-Kramer HSD).

Patterns of pollinator preference and interspecific transitions within foraging bouts

In 2006, *Lythrum* plants received 64–87% of all pollinator visits in mixed-species arrays, significantly more than the expected number of visits (Table 1). Because visits by *B. vagans* were infrequent in 2006, we pooled pollinator species for this analysis. In 2007, *Lythrum* plants again received a large proportion (64–75%) of *B. impatiens* visits. When *Mimulus* and *Lythrum* were equally abundant, there was a significant excess of visits to *Lythrum* by *B. impatiens*, but not by *B. vagans* (Table 2). However, when *Lythrum* was at high relative abundance, there was a significant excess of visits to *Mimulus* plants by both *Bombus* species (Table 2). Visitation to *Lythrum* by *B. vagans* was highly variable (Table 2), and this variability contributed to significant heterogeneity among blocks.

Table 1 The observed and (expected) number of pollinator visits to *Mimulus* and *Lythrum* plants in mixed-species arrays in 2006

Treatment	Number of probes observed		G_P	G_H
	<i>Mimulus</i>	<i>Lythrum</i>		
Equal abundance	71 (98)	125 (98)	15.07*	0.30
<i>Lythrum</i> high abundance	44 (91)	296 (249)	30.32*	1.59

G statistics are presented for data pooled across treatment days (G_P) and for heterogeneity among blocks within each treatment (G_H). Degrees of freedom = 1 for all comparisons

* $P < 0.05$

Interspecific transitions were common in the mixed species arrays during both years, making up 40–56% of all interplant transitions. In some cases, there was a significant excess of interspecific transitions relative to other transition types, but there was no consistent pattern relative to *Lythrum* abundance (Table 3). When foragers sequentially probed *Mimulus*, then *Lythrum*, then *Mimulus*, the number of intervening *Lythrum* flowers probed increased significantly with increasing *Lythrum* abundance ($F_{1,116} = 7.15$, $P < 0.05$). In both years, foragers that made such interspecific transitions probed nearly twice as many intervening *Lythrum* flowers when *Lythrum* was at high relative abundance (Fig. 3).

Effects of *Lythrum* presence and abundance on *Mimulus* reproductive success

The number of seeds per *Mimulus* fruit varied both among competition treatments and among years (Fig. 4, two-way ANOVA, adjusted $R^2 = 0.56$). Both the effect of competition treatment ($F_{2,9} = 4.42$, $P < 0.05$) and the effect of year ($F_{1,9} = 13.79$, $P < 0.005$) were significant. There was no significant interaction between competition treatment and year ($F_{2,9} = 0.11$, $P > 0.90$). In 2006, *Mimulus* seed number per fruit was 32% lower in arrays with an equal frequency of *Lythrum* and *Mimulus* than in arrays without *Lythrum*. When *Lythrum* was at a high relative abundance, *Mimulus* seed number was further reduced (40% lower than in arrays without *Lythrum*). In 2007, *Mimulus* seed number per fruit was 17% lower in arrays with an equal frequency of *Lythrum* and *Mimulus* than in arrays without *Lythrum*. When *Lythrum* was at a high relative abundance, *Mimulus* seed number was further reduced (33% lower than in arrays without *Lythrum*; Fig. 4). In an a posteriori test, *Mimulus* seeds per fruit were found to differ significantly between the arrays with a high relative abundance of *Lythrum* and arrays without *Lythrum* ($P < 0.05$), but the other comparisons were not significant (Fig. 4, Tukey-Kramer HSD). There is a strong directional trend to these data, with increasing *Lythrum* abundance significantly reducing *Mimulus*

Table 2 The observed and (expected) number of *Bombus vagans* and *B. impatiens* visits to *Mimulus* and *Lythrum* plants in mixed-species arrays in 2007

Treatment	Pollinator species	Number of visits		G_P	G_H
		<i>Mimulus</i>	<i>Lythrum</i>		
Equal abundance	<i>B. impatiens</i>	58 (116.5)	175 (116.5)	61.50*	2.21
	<i>B. vagans</i>	74 (68)	62 (68)	1.24	35.35*
<i>Lythrum</i> high abundance	<i>B. impatiens</i>	55 (41)	99 (113)	8.71*	4.97
	<i>B. vagans</i>	53 (32)	67 (88)	51.81*	18.25*

G statistics are presented for data pooled across treatment days (G_P , $df = 1$) and for heterogeneity among blocks within each treatment (G_H , $df = 2$)

* $P < 0.05$

Table 3 The observed and (expected) number of inter-plant pollinator transitions in experimental arrays

Year	Treatment	Transition type observed				G_P	G_H
		<i>M</i> to <i>L</i>	<i>L</i> to <i>M</i>	<i>M</i> to <i>M</i>	<i>L</i> to <i>L</i>		
2006	Equal abundance	19 (17)	17 (17)	26 (10)	12 (30)	34.87*	4.33
	<i>Lythrum</i> high abundance	27 (11)	20 (11)	12 (2)	38 (73)	72.18*	5.29
2007	Equal abundance	47 (34)	41 (34)	32 (16)	36 (72)	38.89*	4.77
	<i>Lythrum</i> high abundance	30 (34)	25 (34)	37 (42)	45 (27)	14.44*	3.63

G statistics are presented for data pooled across treatment days (G_P , $df = 3$) and for heterogeneity among blocks within each treatment (G_H , $df = 3$ for 2006, $df = 6$ for 2007)

M *Mimulus*, *L* *Lythrum*

* $P < 0.05$

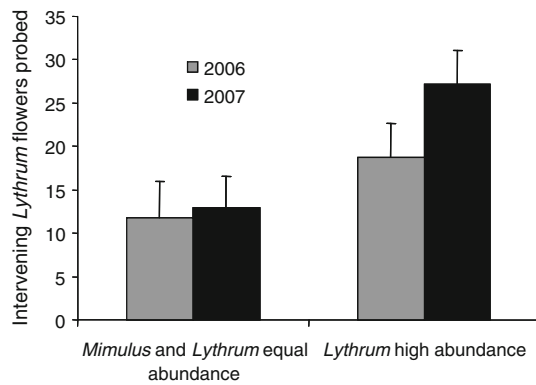


Fig. 3 Mean number (+1 SE) of consecutive *Lythrum* flowers probed per *Mimulus*-to-*Lythrum*-to-*Mimulus* transition in response to experimental manipulation of *Lythrum* abundance

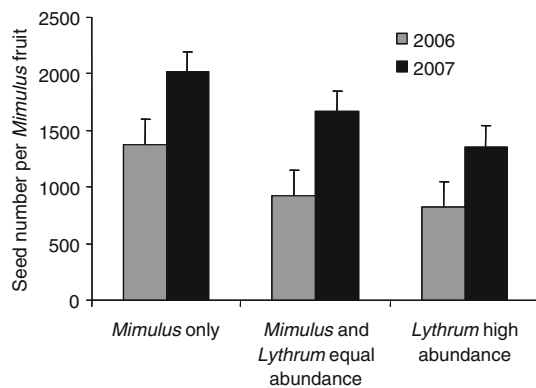


Fig. 4 Effect of *Lythrum* presence and abundance on the mean number of seeds per *Mimulus* fruit (+1 SE)

seed number (ANCOVA, adjusted $R^2 = 0.60$, $F_{1,11} = 7.93$, $P < 0.05$). There were also significant differences among years, but no significant interactions between competition treatment and year.

Discussion

Competition for pollination with invasive exotic *Lythrum salicaria* lowered mean seed number in *Mimulus ringens* fruits. This reduction was most pronounced when invasive *Lythrum* was highly abundant, decreasing the number of seeds per fruit by 40% in 2006 and 33% in 2007. This finding is consistent with the results of a recent meta-analysis (Morales and Traveset 2009) which suggested that an increase in the abundance of invasives may exacerbate the competitive effects that occur at equal species abundances. Here, we explore the mechanisms responsible for this reduction in seeds per fruit and discuss how changes in the relative abundance of invasive competitors may influence the reproduction of native species.

Patterns of pollinator visitation

Exotic invasive species may lower the pollination success of co-occurring native plants if pollinators exhibit preference for the invasive and visit it more frequently than the native species. Through such competition for pollinator preference, the invasive may lower conspecific pollen transfer and seed production of the native species (Waser 1983). Although *Lythrum* is highly attractive to pollinators (Brown et al. 2002), and received an overall higher number of pollinator visits in all contexts, some bees did not exhibit preference for *Lythrum*. For example, in both years, there was a marginally significant trend toward increased visitation to *Mimulus* when *Lythrum* was highly abundant, compared to arrays in which *Lythrum* was present at an equal relative abundance. A positive effect of high *Lythrum* abundance on *Mimulus* visitation rate may have been even more likely in 2007, since the rate of visitation to *Mimulus* when *Lythrum* was abundant was higher than visitation to *Mimulus* in arrays with no *Lythrum* present.

These patterns of pollinator preference and visitation to *Mimulus* may have been influenced by the observed tripling in the number of pollinators recruited to arrays containing *Lythrum* (Fig. 2). Showy invasive species such as *Lythrum salicaria* may act as “magnet species” that increase the overall attractiveness of the patch, recruiting pollinators to a floral stand they would not otherwise forage in (Thomson 1978; Molina-Montenegro et al. 2008). Due to increased pollinator arrivals to arrays, invasives may increase pollinator visitation rates and reproductive success of co-occurring natives if the pollinators do not show perfect preference for the invasive, but rather forage on both species (Rathcke 1983; Johnson et al. 2003; Moeller 2004; Feldman et al. 2004; Ghazoul 2006; Totland et al. 2006; Munoz and Cavieres 2008; Bjercknes et al. 2007). In our study, increased pollinator arrivals to mixed-species arrays may have offset any reduction in *Mimulus* visitation due to pollinator preference for *Lythrum*. Such a scenario may have been especially likely in 2007, when the visitation rate to *Mimulus* increased with a higher relative abundance of *Lythrum*. Our results suggest that the effects of invasives on visitation rates to native species may largely depend on interactions between pollinator preference and changes in patterns of pollinator arrivals to arrays (Bjercknes et al. 2007; Lopezaraiza-Mikel et al. 2007; Munoz and Cavieres 2008; Mitchell et al. 2009a).

Effects of visit quality on pollen deposition and seed production

Pollinator movement between native and invasive species can strongly influence patterns of pollen transfer, and may lower the reproductive success of native species, even if

visitation rate is not affected (Waser 1983). Interspecific transitions were very common in all mixed species arrays during both years, making up 40–56% of all interplant transitions. Although there was no consistent pattern with respect to *Lythrum* abundance, the high proportion of interspecific pollinator movement in the mixed-species arrays suggests that there is strong potential for interspecific pollen transfer to lower reproductive success of the native species.

Frequent pollinator movements between a focal species and a competitor are likely to promote pollen loss, which may be magnified with increasing relative abundance of the competitor (Morales and Traveset 2008). Although the proportion of interspecific transitions did not vary with *Lythrum* abundance, the number of *Lythrum* flowers probed per interspecific transition did vary with *Lythrum* abundance, which could magnify *Mimulus* pollen loss. When *Lythrum* was abundant, inconstant foragers departing *Mimulus* sequentially probed nearly twice as many *Lythrum* flowers before returning to *Mimulus* (Fig. 3). Pollen loss may result from pollinator contact with the stigmas or other floral surfaces of competitor flowers (Campbell 1985; Feinsinger et al. 1988; Feinsinger and Tiebout 1991; Murcia and Feinsinger 1996; Morales and Traveset 2008) or from grooming or other transport-related losses that occur while the pollinator forages (Thomson 1986; Inouye et al. 1994; Harder and Wilson 1998; Flanagan et al. 2009; Mitchell et al. 2009a). Following these losses, when a pollinator returns to flowers of a focal species, fewer conspecific pollen grains may remain available for deposition onto stigmas of the focal species, potentially leading to a reduction in *Mimulus* reproductive success (Flanagan et al. 2009).

The reproductive success of native species may also be lowered if pollinators deposit pollen grains of the invasive onto the stigma of the native species, blocking or clogging the stigmatic surface or interfering with the successful germination of conspecific pollen (Waser 1978; Waser and Fugate 1986; Galen and Gregory 1989; Caruso and Alfaro 2000; Brown and Mitchell 2001). However, it is also possible that the presence of heterospecific grains will have little influence on seed number (Campbell and Motten 1985; Murcia and Feinsinger 1996; Jakobsson et al. 2008). Pollen of the two plant species is partitioned on the pollinator's body, such that little contact is made between *Lythrum* pollen carried by the bee and *Mimulus* stigmas. Additionally, in an earlier study (Flanagan et al. 2009), we showed that very few *Lythrum* grains were deposited on *Mimulus* stigmas by bees carrying *Lythrum* pollen, and that the presence of *Lythrum* pollen on *Mimulus* stigmas did not influence seed number. Therefore, inbound interspecific pollen transfer is unlikely to be responsible for the observed reductions in *Mimulus* reproductive success. Rather, we think reductions in *Mimulus* seed set are due to pollen loss during

interspecific visits to *Lythrum*. In particular, we think these losses may be due to pollinator grooming and passive transport-related loss during interspecific transitions by bumblebees (Flanagan et al. 2009). This result is in contrast to Brown and Mitchell's (2001) finding that deposition of *Lythrum salicaria* grains on stigmas of native *Lythrum alatum* lowered *L. alatum* seed set.

Seed number in *Mimulus* fruits varied markedly among years. This finding may reflect changes in the composition of bumblebee species probing *Mimulus* in 2006 and 2007 and differences in their preference for *Lythrum* versus *Mimulus*. Although the presence of *Lythrum* lowered *Mimulus* seed number in both years, the striking among-year variation in seeds per fruit emphasizes the importance of studying competition for pollination in multiple years (Larson et al. 2006).

A number of factors relating to plant size and vigor, resource limitation, or other abiotic or biotic environmental conditions could also contribute to the observed among-year variation in seed number (Klinkhamer and DeJong 1987; DiFazio et al. 1998; Griffin and Barrett 2002; Shimono and Washitani 2007). In particular, one weakness of our study is the absence of a supplemental hand-pollination treatment (Mitchell et al. 2009b). However, the use of potted plants allowed us to have a high level of control over the resource status of plants used in this experiment (see "Materials and methods"). Therefore, we feel confident that only the competitive background experienced by plants varied, and that differences in seed set among treatment groups were due to differences in pollination, rather than to differences in resource status.

Conclusion

Invasive species often have large populations, and their abundance relative to co-occurring natives may influence the outcome of competition for pollination. We found that the presence of invasive *Lythrum salicaria* increased the number of pollinators visiting experimental arrays, but did not lower the visitation rate to *Mimulus ringens*. However, the presence of *Lythrum* lowered mean seed number in *Mimulus* fruits, with reductions most pronounced when the invasive was highly abundant. We attribute much of this reduction in seed number to pollen loss when bees carrying *Mimulus* pollen probed intervening *Lythrum* flowers. Although the proportion of interspecific transitions made by pollinators was not influenced by *Lythrum* abundance, an increase in *Lythrum* abundance led to a doubling in the number of intervening *Lythrum* flowers probed, potentially magnifying pollen loss. This study emphasizes the importance of studying competition for pollination across a range of competitor abundances, and demonstrates that visit quality,

not just visit number, may be an important determinant of the effects of exotic, invasive species on the reproductive success of native species.

Acknowledgments The authors thank T. Schuck for assistance propagating *Mimulus ringens* and J. Reinartz, G. Meyer, and L. Nelson for help with field work. We thank S. Johnson, O. Totland, and an anonymous reviewer for helpful comments on an earlier draft of this manuscript. This research was supported by a grant from Applied Ecological Services to R.J.F. and grants from the National Science Foundation to J.D.K. (DEB 9816712) and R.J.M. (DEB 9903308). This experiment complies with the current laws of the United States of America.

References

- Agren J (1996) Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* 77:1779–1790
- Bell JM, Karron JD, Mitchell RJ (2005) Interspecific competition for pollination lowers seed production and outcrossing rate in *Mimulus ringens*. *Ecology* 86:762–771
- Bjerknes AL, Totland O, Hegland SJ, Nielsen A (2007) Do alien plant invasions really affect pollination success in native plant species? *Biol Conserv* 138:1–12
- Blossey BL, Skinner O, Taylor J (2001) Impact and management of purple loosestrife (*Lythrum salicaria*) in North America. *Biodivers Conserv* 10:1787–1807
- Brown BJ, Mitchell RJ (2001) Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. *Oecologia* 129:43–49
- Brown BJ, Mitchell RJ, Graham SA (2002) Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology* 83:2328–2336
- Campbell DR (1985) Pollinator sharing and seed set of *Stellaria pubera*: competition for pollination. *Ecology* 66:544–563
- Campbell DR, Motten AF (1985) The mechanism of competition for pollination between two forest herbs. *Ecology* 66:554–563
- Caruso CM (2002) Influence of plant abundance on pollination and selection on floral traits of *Ipomopsis aggregata*. *Ecology* 83:241–254
- Caruso CM, Alfaro M (2000) Interspecific pollen transfer as a mechanism of competition: effect of *Castilleja linariaefolia* pollen on seed set of *Ipomopsis aggregata*. *Can J Bot* 78:600–606
- Chittka L, Schurkens S (2001) Successful invasion of a floral market. *Nature* 411:653
- Dafni A, Kevan PG, Husband BC (eds) (2005) Practical pollination biology. *Enviroquest*, Cambridge
- Darwin C (1877) The different forms of flowers on plants of the same species. *Appleton*, New York
- DiFazio SP, Wilson MV, Vance NC (1998) Factors limiting seed production of *Taxus brevifolia* (Taxaceae) in western Oregon. *Am J Bot* 85:910–918
- Farnsworth EJ, Ellis DR (2001) Is purple loosestrife (*Lythrum salicaria*) an invasive threat to freshwater wetlands? *Conflicting evidence*. *Wetlands* 21:199–209
- Feinsinger P, Tiebout HM III (1991) Competition among plants sharing hummingbird pollinators: laboratory experiments on a mechanism. *Ecology* 72:1946–1952
- Feinsinger P, Busby WH, Tiebout HM III (1988) Effects of indiscriminate foraging by tropical hummingbirds on pollination and plant reproductive success: experiments with two tropical treelets (Rubiaceae). *Oecologia* 76:471–474
- Feldman TS, Morris WF, Wilson WG (2004) When can two plants facilitate each other's pollination? *Oikos* 105:197–207
- Flanagan RJ, Mitchell RJ, Knutowski D, Karron JD (2009) Interspecific pollinator movements reduce pollen deposition and seed production in *Mimulus ringens* (Phrymaceae). *Am J Bot* 96:809–815
- Galen C, Gregory T (1989) Interspecific pollen transfer as a mechanism of competition: consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. *Oecologia* 81:120–123
- 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
- Grant AL (1924) A monograph of the genus *M. ringens*. *Ann M Bot Gard* 11:99–389
- Griffin SR, Barrett SCH (2002) Factors affecting low seed-ovule ratios in a spring woodland herb, *Trillium grandiflorum* (Melanthiaceae). *Int J Plant Sci* 163:581–590
- Hager H, McCoy KD (1998) The implications of accepting untested hypotheses: a review of the effects of purple loosestrife (*Lythrum salicaria*) in North America. *Biodivers Conserv* 7:1069–1079
- Harder LD, Wilson WG (1998) Theoretical consequences of heterogeneous transport conditions for pollen dispersal by animals. *Ecology* 79:2789–2807
- Husband BC, Barrett SCH (1992) Pollinator visitation in populations of tristylous *Eichhornia paniculata* in northeastern Brazil. *Oecologia* 89:365–371
- Inouye DW, Gill DE, Dudash MR, Fenster CB (1994) A model and lexicon for pollen fate. *Am J Bot* 81:1517–1530
- Ippolito A, Fernandes GW, Holtsford TP (2004) Pollinator preferences for *Nicotiana glauca*, *N. glauca*, and their F₁ Hybrids. *Evolution* 58:2634–2644
- Jakobsson A, Padron B, Traveset A (2008) Pollen transfer from invasive *Carpobrotus* spp. to natives—a study of pollinator behaviour and reproduction success. *Biol Conserv* 141:136–145
- Johnson SD, Peter CI, Nilsson LA, Agren J (2003) Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology* 84:2919–2927
- Kandori I, Hirao T, Matsunaga S, Kurosaki T (2009) An invasive dandelion unilaterally reduces the reproduction of a native congener through competition for pollination. *Oecologia* 159:559–569
- Karron JD, Thumser NN, Tucker R, Hesseauer AJ (1995a) The influence of population density on outcrossing rates in *Mimulus ringens*. *Heredity* 75:175–180
- Karron JD, Tucker R, Thumser NN, Reinartz JA (1995b) Comparison of pollinator flight movements and gene dispersal patterns in *Mimulus ringens*. *Heredity* 75:612–617
- Karron JD, Jackson RT, Thumser NN, Schlicht SL (1997) Outcrossing rates of individual *Mimulus ringens* genets are correlated with anther–stigma separation. *Heredity* 79:365–370
- Karron JD, Mitchell RJ, Holmquist KG, Bell JM, Funk B (2004) The influence of floral display size on selfing rates in *Mimulus ringens*. *Heredity* 92:242–248
- Karron JD, Mitchell RJ, Bell JM (2006) Multiple pollinator visits to *Mimulus ringens* (Phrymaceae) flowers increase mate number and seed set within fruits. *Am J Bot* 93:1306–1312
- Karron JD, Holmquist K, Flanagan RJ, Mitchell RJ (2009) Pollinator visitation patterns strongly influence among-flower variation in selfing rate. *Ann Bot* 103:1379–1383
- Klinkhamer PGL, DeJong TJ (1987) Plant size and seed production in the monocarpic perennial *Cynoglossum officinale* L. *New Phytol* 106:773–783
- Larson DL, Royer RA, Royer MR (2006) Insect visitation and pollen deposition in an invaded prairie plant community. *Biol Conserv* 130:148–159

- Lopezaraiza-Mikel ME, Hayes RB, Whalley R, Memmott J (2007) The impact of an alien plant on a native plant-pollinator network: an experimental approach. *Ecol Lett* 10:539–550
- Mal TK, Lovett-Doust J, Lovett-Doust L, Mulligan GA (1992) The biology of Canadian weeds. 100. *Lythrum salicaria*. *Can J Plant Sci* 72:1305–1330
- Mitchell RJ, Karron JD, Holmquist KG, Bell JM (2004) The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. *Funct Ecol* 18:116–124
- Mitchell RJ, Karron JD, Holmquist KG, Bell JM (2005) Patterns of multiple paternity in fruits of *Mimulus ringens* (Phrymaceae). *Am J Bot* 92:885–890
- Mitchell RJ, Flanagan RJ, Brown BJ, Waser NM, Karron JD (2009a) New frontiers in competition for pollination. *Ann Bot* 103:1403–1413
- Mitchell RJ, Irwin RE, Flanagan RJ, Karron JD (2009b) Ecology and evolution of plant-pollinator interactions. *Ann Bot* 103:1355–1363
- Moeller DA (2004) Facilitative interactions among plants via shared pollinators. *Ecology* 85:3289–3301
- Molina-Montenegro MA, Badano EI, Cavieres LA (2008) Positive interactions among plant species for pollinator service: assessing the ‘magnet species’ concept with invasive species. *Oikos* 117:1833–1839
- Morales CL, Aizen MA (2006) Invasive mutualisms and the structure of plant-pollinator interactions in the temperate forests of north-west Patagonia, Argentina. *J Ecol* 94:171–180
- Morales CL, Traveset A (2008) Interspecific pollen transfer: magnitude, prevalence, and consequences for plant fitness. *Crit Rev Plant Sci* 27:221–238
- Morales CL, Traveset A (2009) A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. *Ecol Lett* 12:716–728
- Munoz AA, Cavieres LA (2008) The presence of a showy invasive plant disrupts pollinator service and reproductive output in native alpine species only at high densities. *J Ecol* 96:459–467
- Murcia C, Feinsinger P (1996) Interspecific pollen loss by hummingbirds visiting flower mixtures: effects of floral architecture. *Ecology* 77:550–560
- Newman JA, Bergelson J J, Grafen A (1997) Blocking factors and hypothesis tests in ecology: is your statistics text wrong? *Ecology* 78:1312–1320
- Rathcke B (1983) Competition and facilitation among plants for pollination. In: Real L (ed) *Pollination biology*. Academic, Orlando, pp 305–309
- SAS Institute (2004) JMP version 5.1.2 statistical software. SAS Institute, Cary
- Shimono A, Washitani I (2007) Factors affecting variation in seed production in the heterostylous herb *Primula modesta*. *Plant Species Biol* 22:65–76
- Sokal RR, Rohlf FJ (1995) *Biometry: the principles and practice of statistics in biological research*, 3rd edn. Freeman, New York
- Takakura K, Nishida T, Matsumoto T, Nishida S (2009) Alien dandelion reduces the seed-set of a native congener through frequency-dependent and one-sided effects. *Biol Invasions* 11:973–981
- Thompson DQ, Stuckey RL, Thompson EB (1987) Spread, impact and control of purple loosestrife in North American wetlands. US Fish and Wildlife Research, Washington DC
- Thomson JD (1978) Effect of stand composition on insect visitation in two-species mixtures of *Hieracium*. *Am Midl Nat* 100:431–440
- Thomson JD (1986) Pollen transport and deposition by bumble bees in *Erythronium*: influences of floral nectar and bee grooming. *J Ecol* 74:329–341
- Totland O, Nielsen A, Bjerknes AJ, Ohlson M (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
- Waser NM (1978) Competition for pollination and sequential flowering in two Colorado wildflowers. *Ecology* 59:934–944
- Waser NM (1983) Competition for pollination and floral character differences among sympatric plant species: a review of evidence. In: Jones CE, Little RJ (eds) *Handbook of experimental pollination biology*. Van Nostrand Reinhold, New York, pp 277–293
- Waser NM, Fugate ML (1986) Pollen precedence and stigma closure: a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. *Oecologia* 70:573–577