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## Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener

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**Abstract** Competition for pollination can be an important factor in plant reproduction, but little attention has been given to the effect of the growing number of invasive plant species on pollination of native species. As a first step in understanding this threat, we used hand pollination to investigate the effects of pollen from an invasive species (*Lythrum salicaria*) on seed set in a sympatric and co-flowering native congener (*L. alatum*). Dispersal of fluorescent dyes in the field confirms that pollinators (bumble bees and honey bees) transfer pollen between species. To determine the potential effect of such interspecific pollen transfer on seed set of the native, we pollinated 773 flowers on 20 plants with one of three treatments: legitimate conspecific pollen, a mixture of conspecific and foreign pollen, and foreign pollen. The mixed-pollen treatment resulted in 28.8% lower seed set relative to conspecific pollination. Foreign crosses resulted in extremely low seed set. Observations of pollen germination indicate that events at the stigmatic surface contribute to the reduction in seed set for mixed pollination. Our results indicate that the impacts of invasive species may extend beyond vegetative competition to include competition for pollination.

**Keywords** Dye transfer · Heterostyly · Hybridization · Interspecific pollen · *Lythrum*

### Introduction

Invasive plant species are frequently considered excellent competitors for resources, but rarely has their impact on competition for pollinator services been considered. Such competition could occur through changes in pollinator behavior and result in reduced visitation (reduced pollen quantity), or in deposition of heterospecific pollen (reduced pollen quality).

Flowers often receive pollen from other species because of the inconstant foraging behavior of pollinators (Waser 1978, 1983; Ganders 1979; Schemske 1981; Rathcke 1983; Feinsinger et al. 1986; Jennersten et al. 1988; Arroyo and Dafni 1993; Murphy and Aarssen 1995a; McLernon et al. 1996). Such interspecific pollen transfer (IPT; Waser 1983, also Rathcke 1983) has been recognized as an important mechanism through which plants can compete for pollination services (Waser 1983; Rathcke 1983), in part because it can impair seed set even in the absence of pollinator limitation (Waser 1983; Randall and Hilu 1990; Petanidou et al. 1995; Bergman 1996). IPT can reduce seed production in several ways, including pollen allelopathy (Char 1977; Sukhada and Jayachandra 1980; Thomson et al. 1981; Murphy and Aarssen 1995a, b, c, d), stigma clogging (Shore and Barrett 1984; Galen and Gregory 1989; Proctor et al. 1996), stigma closing (Waser and Fugate 1986), stylar clogging (Palmer et al. 1989; Scribailo and Barrett 1994), and stylar inhibition (Williams et al. 1982; Cruzan 1990, 1993). Even when IPT does not reduce seed set (Schemske 1981; Shore and Barrett 1984; Kohn and Waser 1985; Kwak and Jennersten 1986; Galen and Gregory 1989; McGuire and Armbruster 1991), it may reduce male fitness (Waser 1978; Campbell and Motten 1985; Feinsinger et al. 1986; Jennersten and Kwak 1991; McGuire and Armbruster 1991) through pollen loss. Furthermore, when co-flowering plants are closely related, IPT may also cause interspecific gene flow and hybridization (Scheffler and Dale 1994; Bing et al. 1996; Brown and Brown 1996; Bartsch et al. 1999; Ellstrand et al. 1999).

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Most studies of competition for pollination have focused on interactions between native species (e.g., Campbell and Motten 1983, 1985; Waser 1983; Feinsinger et al. 1986; Galen and Gregory 1989; McGuire and Armbruster 1991; but see Free 1970). However, the increasing worldwide incidence of invasion by alien plant species (Wilcove et al. 1998) raises the possibility that pollination of natives might suffer when they are sympatric with one of the many invasives. Invasives could affect pollination of both wind-pollinated (through reduction in pollen quality) and animal-pollinated plants (through reduction in pollen quantity and pollen quality). Where invasives are abundant, their competitive effects on natives may extend a considerable distance, since pollinators may forage over large distances and wind may disperse pollen widely. This is in contrast to the situation for vegetative competition, which requires quite close proximity, and has been much more heavily studied. More studies on the effects of invasive species on pollination of natives are required before the severity and extent of this potential threat can be properly evaluated.

We studied the effect of the invasive *Lythrum salicaria* (purple loosestrife) on pollination of native *L. alatum* (winged loosestrife). These two animal-pollinated species are well suited for this work because they flower synchronously and in close sympatry, share pollinators, and have similar floral morphologies. Furthermore, the invasive *L. salicaria* produces large and rewarding flowers, and is heavily visited by pollinators, indicating that it has the potential to affect pollination of other native North American species in addition to *L. alatum*.

In this paper we specifically address two questions: (1) Does pollen from the invasive *L. salicaria* reach stigmas of the native species in the field? (2) Does the presence of foreign pollen from *L. salicaria* reduce seed set in *L. alatum*? We also explore possible mechanisms for the observed reduction in seed set. Elsewhere we document effects on visitation rates and seed set in field populations and in artificial arrays of these species (Brown 1999).

## Materials and methods

### Experimental organisms

*Lythrum salicaria* L. (Lythraceae) is an invasive Eurasian perennial up to 2 m in height which invades wetlands, creating floral monocultures and reducing faunal diversity (Thompson et al. 1987; Mal et al. 1992). The large, tristylous flowers are purple to rose-purple, and form showy terminal spikes (Graham 1975). *Lythrum alatum* Pursh is the most widespread native species of *Lythrum* in the United States. It is a perennial up to 1 m in height, commonly found in the eastern United States, and much less showy than *L. salicaria*. The small, distylous flowers are light to medium purple and occur singly or in pairs in the leaf axils (Graham 1975).

*L. salicaria* and *L. alatum* are both wetland species, and although *L. salicaria* is found in slightly moister habitats there can be extensive areas of overlap (up to several hectares; B. Brown, personal observation) where the species co-occur. In these areas of

**Table 1** Mixed model ANOVA for seed set after hand pollination with legitimate *Lythrum alatum* or mixed (*L. alatum* + *L. salicaria*) pollen load; model  $R^2=0.649$ . Because Plant (Morph) is a random factor, we tested Treatment, Plant (Morph) and Treatment  $\times$  Morph over Error, while Morph is tested over Plant (Morph)

Source	df	MS	F	P
Treatment	2	3,731	19.32	<0.0001
Morph	1	665	2.36	0.13
Plant (Morph)	18	342	1.77	0.09
Treatment $\times$ Morph	2	37	0.19	0.8
Error	25	193		

sympatry, the two species are frequently within 1 m of each other (e.g., mean nearest neighbor distance between these species of  $0.58 \pm 0.12$  m,  $n=15$  at Ottawa National Wildlife Refuge, Ohio, USA, random sample along a transect at the interface between the two population). The species are sympatric throughout most of their northern range and flower simultaneously for 6–8 weeks. During the blooming period *L. alatum* plants average  $16 \pm 3.3$  open flowers (mean  $\pm$  SE,  $n=26$ ) whereas *L. salicaria* averages  $120 \pm 29.2$  flowers ( $n=31$ ). The flowers of both species are similar in shape and color, although individual flowers of *L. salicaria* are substantially larger than are those of *L. alatum* (mean + SE petal length for *L. salicaria* =  $8.71 \pm 0.26$ ,  $n=47$ ; for *L. alatum* =  $2.23 \pm 0.16$ ,  $n=46$ ). In field populations there is nearly complete overlap in the pollinator fauna of these two species – most visits are by bumble bees and honey bees, both of which move freely between the two species (Levin 1970; Brown 1999).

Both species are heterostylous, with stigma and stamens at different heights within a flower, but one floral morph per plant. *L. salicaria* is tristylous with flowers on a plant exhibiting one of three stigma heights (short, mid and long) and two whorls of stamens. *L. alatum* is distylous with two stigma heights (short and long) and one whorl of stamens. Both species exhibit other heterostylous morphological characters, including morph-specific pollen size (Darwin 1877) and stigmatic papillae length (B. Brown, unpublished data). Most heterostylous species, including *L. salicaria*, produce no seeds when crossed with plants of the same morph, but do produce seeds when crossed with plants of another morph (Barrett 1992; O'Neil 1994), such that maximum seed set requires legitimate pollen from another plant of a different morph (Darwin 1877). Following the terminology of Cruzan and Barrett (1993) we use the term "legitimate pollination" to refer to pollen transfer from anthers at the same height as the stigma. Within-morph incompatibility and between-morph compatibility are suspected but unconfirmed in *L. alatum*.

### Foreign pollen

Seeds collected from populations at Ottawa National Wildlife Refuge (Ottawa County, Ohio, USA) in 1996 were stratified for 3 months and grown in the greenhouse in spring 1997. From these plants, we selected 40 *L. alatum* and 15 *L. salicaria* (equal morph frequencies for each species). The 15 *L. salicaria* and 20 of the *L. alatum* were designated pollen donors. The remaining 20 *L. alatum* were pollen recipients. Entire plants were bagged and maintained in the greenhouse during the experiment.

For 5 weeks beginning 23 July 1997 we used toothpicks to pollinate open flowers with one of the following three treatments: legitimate *L. alatum* pollen (all *L. alatum* pollen), mixed (equal numbers of anthers from each species), foreign (all *L. salicaria* pollen). Pollen for the legitimate mixture was obtained by removing 12 anthers each from 10 plants of the legitimate morph (a total of 120 anthers). We obtained pollen for the mixed pollen application by removing 6 anthers from each of 10 plants of *L. alatum* and 6 anthers from each of 9 *L. salicaria* (3 plants of each morph; 114 anthers in total across species). The foreign mixture was obtained by removing 12 anthers

from each of 9 *L. salicaria*, (3 plants of each morph; 108 anthers). Anthers were placed in 1.5-ml microcentrifuge tubes, exposed to 60 W incandescent light at 8 cm for 30 min to allow anthers to de-hisce fully and were stirred with tooth picks to facilitate complete mixing of the pollen prior to application. Pollen production per flower for *L. salicaria* is twice that of *L. alatum* (respectively, mean + SE = 11,454 + 1,333,  $n=22$ ; vs 5,577 + 654,  $n=38$ ); pollen production does not differ significantly among morphs within either species. Pollen from the two species is indistinguishable because of similarity in structure and size. These pollination methods typically deposited 400–500 pollen grains on stigmas, an amount that should be sufficient for full *L. alatum* seed set even for the mixed treatment (personal observation).

All open flowers on treatment days received one of the three treatments. We emasculated short-morph flowers just before hand-pollination, to reduce the amount of self-pollen they received. Treatments were indicated by color-coded acrylic paint applied to the calyx. We collected fruits when mature and determined seed set using a dissecting scope at 6 $\times$ .

We used mixed-model ANOVA in SAS (SAS Institute, Cary, N.C.) to analyze variation in seed set/fruit (number of seeds/developed fruit) and proportion fruit set (no. of developed fruits/no. of pollinated flowers). Developed fruits are any capsules containing mature seeds. To avoid pseudoreplication, we used means for each plant-treatment combination as the unit of sampling. All residuals were normally distributed and showed no significant heteroscedasticity. We considered effects of treatment and morph to be fixed, and effects of plant nested within morph to be random, and therefore derived denominator *MS* for *F* tests following Winer (1971; see Table 1 for specifics).

From a total of 773 flowers pollinated (long 378: 128 legitimate, 132 mixed, 118 foreign; short 395: 136 legitimate, 131 mixed, 128 foreign), 605 were recovered. The remaining fruits presumably had zero seed set since mature fruits are very difficult to remove from a branch, while those with no seed set readily detach (personal observation, B. Brown and S. Graham, Kent State University, Kent, Ohio).

#### Dye transfer

During August 1998, we used three *L. salicaria* plants (one of each morph) to determine if pollen moved between *L. alatum* and *L. salicaria*. Because their pollen is indistinguishable, we used fluorescent orange dye particles (Radiant Color, Richmond, Calif.) as a pollen analog for *L. salicaria* to track pollen transfer in arrays of potted plants (Waser 1988; O'Neil 1992). For each array, four potted *L. alatum* plants (two plants per morph) were placed at the corners of a 1 m square surrounding a single *L. salicaria* plant. There were a total of three arrays, one for each *L. salicaria* morph. Using a toothpick we placed dye on all anthers of 30 flowers on the *L. salicaria* plant. We took great care to prevent contamination of *L. alatum* while applying dye to *L. salicaria*, for example, changing clothes and thoroughly washing hands and arms before harvesting *L. alatum*. Other *Lythrum* plants in the vicinity were at least 5 m distant from the experiment. After 3 h, we collected all open *L. alatum* flowers and placed them in individual microcentrifuge tubes. Within 24 h, we examined each stigma for the presence and number of dye particles using a dissecting scope at 50 $\times$ . We repeated this procedure on 3 consecutive days, rotating the *L. salicaria* between the three sets of *L. alatum*, for a total of 9 array-days. We analyzed the data using ANOVA. We considered individual recipient plants on each day as our unit of sampling, and therefore analyzed mean number of dye particles per flower, and proportion of flowers with dye for each plant on each day. Number of particles present was square root transformed, while it was not necessary to transform the percentage of stigmas receiving dye.

#### Pollen tube growth

In August 1998, we randomly selected eight long- and eight short-styled *L. alatum* plants from those available. Ten of these plants

were designated pollen recipients (five short- and five long-styled plants) while the remaining six were designated pollen donors. We also chose six *L. salicaria* plants (two of each morph) to serve as pollen donors. Each species was represented by equal morph frequencies and all plants were bagged 24 h prior to the beginning of the experiment. We applied one of three pollen treatments to recipient plants using toothpicks: 100% legitimate, 100% foreign, and no pollen. We obtained pollen for the legitimate treatment by removing anthers from two flowers from each donor plant (six flowers per morph). For the foreign pollen treatment we removed anthers from one flower on each *L. salicaria* morph, or the equivalent of two flowers per morph. This procedure was repeated twice, once for each morph of *L. alatum*. All pollen mixtures were treated as above.

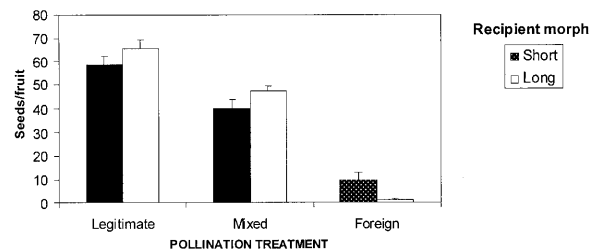
On each plant 4–6 flowers received each treatment. As above, we emasculated short-morph flowers prior to pollination. Twenty-four hours after the initial application of pollen, we harvested 2–3 flowers and removed the pistil and placed it in a 1.5-ml microcentrifuge tube and added 1.5 ml FAA preservative (Dafni 1992) for 24 h, then replaced it with 70% ethanol. We replicated this experiment twice in late August 1998. After staining with aniline blue dye (Dafni 1992) we viewed pistils under epifluorescence microscopy and counted the total number of pollen tubes that had grown to the base of the style. Because pollen and pollen tubes of the two species cannot be distinguished, counts are totals of pollen tubes for that flower.

We used ANOVA on plant means for each treatment to test for variation in 24-h pollen tube growth as a response to treatment, morph, plant (morph) and the interaction of treatment and morph. All residuals were normally distributed but showed significant heteroscedasticity. However, log transformations equalized variances among treatment-morph combinations. In the interest of clarity and simplicity we present the untransformed analysis here, but patterns of statistical significance are identical for the log transformed analyses.

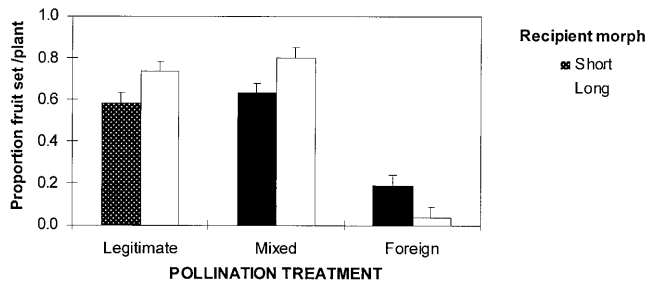
## Results

### Foreign pollen

Pollination with a mixture of legitimate and foreign pollen reduced *L. alatum* seed set by 28.8%, compared to legitimate pollination (Fig. 1), while seed set in the foreign treatment was extremely low (see below). Pollination treatment significantly affected seed set for *L. alatum* (Table 1), and a priori contrasts indicate that seed set in the legitimate treatment is significantly higher than in the mixed or foreign treatments ( $F_{1, 25}=14.2$ ,  $P<0.001$ , and  $F_{1, 25}=34.4$ ,  $P<0.0001$  respectively). Seed set did not



**Fig. 1** Seed set by morph following pollen treatments: *Legitimate*=100% legitimate *Lythrum alatum*, *Mixed*=*L. salicaria* and *L. alatum*, *Foreign*=100% *L. salicaria* pollen. Values shown are means + SE. For each bar  $n=10$  plants, except for the foreign treatment where  $n=2$  for long morph and  $n=7$  for short morph, 389 fruits total



**Fig. 2** Proportion fruit set per plant by morph following pollen treatments: *Legitimate*=100% legitimate *L. alatum*, *Mixed*=*L. alatum* and *L. salicaria*, *Foreign*=100% *L. salicaria* pollen. Values shown are means + SE.  $n=10$  plants per bar

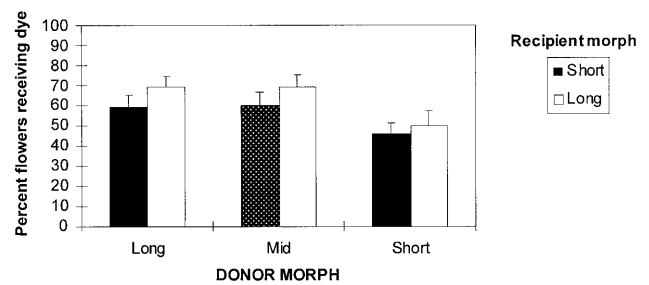
**Table 2** Mixed model ANOVA for proportion fruit set after hand pollination with legitimate *L. alatum* or mixed (*L. alatum* + *L. salicaria*) pollen loads; model  $R^2=0.875$ . Denominator *MS* as in Table 1

Source	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Treatment	2	2.18	91.49	<0.0001
Morph	1	0.04	0.58	0.46
Plant (Morph)	18	0.07	2.98	0.003
Treatment × Morph	2	0.16	6.81	0.003
Error	36	0.02		

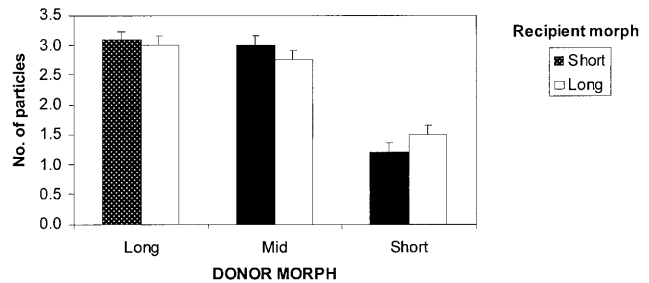
vary significantly among morphs, plants, or morph treatment combinations. Pollination with only foreign pollen resulted in low, but non-zero seed set per fruit (long morph 1.33 seeds/fruit; short morph 10.04 seeds/fruit), indicating the potential for limited hybridization (Fig. 1). Note, however, that seed set for the foreign pollen treatment occurred almost exclusively in the short morph (98.4% of 245 putative hybrid seeds). Fruit set varied significantly with treatment and among plants within morphs (Table 2, Fig. 2). The interaction of treatment and morph was significant, primarily because the foreign pollen treatment caused a reversal in the rank performance of the two morphs (Fig. 2). A planned contrast indicates that there was no significant difference in fruit set between the legitimate and mixed treatments ( $F_{1,36}=1.48$ ,  $P=0.23$ ), but that there was a significant reduction for the foreign treatment ( $F_{1,36}=150$ ,  $P<00001$ ).

#### Dye transfer

Dye commonly moved from *L. salicaria* to *L. alatum* in our arrays; overall, we found dye on 63.3% of the *L. alatum* flowers (Fig. 3) after only 3 h of exposure to pollinators. Despite the high proportion of *L. alatum* flowers receiving dye from *L. salicaria*, those flowers received relatively few particles of dye during 3 h exposure (Fig. 4). In both analyses, recipient morphs did not vary, nor did donor morphs (Tables 3, 4), and the only significant source of variation was replicate, indicating significant variation in dye dispersal among days.



**Fig. 3** Percent of *L. alatum* flowers receiving fluorescent dye from *L. salicaria* after 3 h. Values shown are means + SE.  $n=6$  plants-day/bar



**Fig. 4** Number of fluorescent dye particles received by *L. alatum* flowers from *L. salicaria* after 3 h. Values shown are means + SE.  $n=6$  plants-day/bar

**Table 3** ANOVA for percentage of *L. alatum* flowers/plant receiving dye; model  $R^2=0.155$

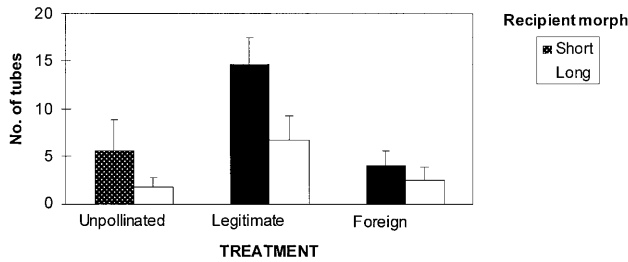
Source	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Recipient morph	1	0.23	3.29	0.08
Donor morph	2	0.09	1.21	0.31
Replicate	2	0.23	3.29	0.052
Recipient morph × Donor morph	2	0.04	0.55	0.59
Error	28	0.07		

**Table 4** ANOVA for the number of dye particles on *L. alatum* flowers after 3 h; model  $R^2=0.127$

Source	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Recipient morph	1	0.31	0.80	0.38
Donor morph	2	1.08	2.72	0.08
Replicate	2	1.70	4.30	0.02
Recipient morph × Donor morph	2	0.16	0.39	0.70
Error	28	0.40		

#### Pollen tube growth

The number of pollen tubes reaching the bottom of the style after 24 h varied among treatments and morphs (Fig. 5, Table 5), with conspecific pollen producing three times more tubes than in the unpollinated controls. Planned contrasts revealed no significant difference in the number of pollen tubes for unpollinated control flow-



**Fig. 5** Number of pollen tubes at the bottom of styles of *L. alatum* 24 h after receiving one of three pollen treatments: *Unpollinated*, *Legitimate*=100% legitimate, or *Foreign*=100% *L. salicaria*. Values shown are means + SE.  $n=10$  plants/bar

**Table 5** Mixed model ANOVA for number of pollen tubes at the bottom of styles of *L. alatum* 24 h after pollination with *L. alatum* pollen, *L. salicaria* pollen, or unpollinated; model  $R^2=0.749$ . Denominator *MS* as in Table 1

Source	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Treatment	2	118.2	4.23	0.03
Morph	1	220.4	6.52	0.03
Plant (Morph)	8	33.8	1.21	0.35
Treatment×Morph	2	45.6	1.63	0.23
Error	16	27.9		

ers and those pollinated with pollen of *L. salicaria* ( $F_{1,16}=2.15$ ,  $P>0.16$ ), but significantly more tubes for flowers pollinated with *L. alatum* pollen compared to those pollinated with *L. salicaria* ( $F_{1,16}=8.5$ ,  $P<0.01$ ). Despite our efforts to prevent contamination, the unpollinated flowers occasionally had pollen tubes. This raises the possibility that the tubes found in the foreign treatment are not from *L. salicaria*, but instead result from self-pollination. This reinforces our conclusion that *L. salicaria* pollen tubes are less successful than are legitimate conspecific pollen.

## Discussion

We found that *L. salicaria* pollen can substantially decrease seed set in *L. alatum* under our experimental conditions and that such foreign pollen transfer may be common in the field. This result, along with work in field populations (Brown 1999), suggests that the potential for invasives to out-compete native species may be magnified by the effect of competition for pollination through IPT, deepening the danger of invasives to the native species with which they share pollinators or pollination agents.

### Foreign pollen

The reduction in seed set experienced by *L. alatum* with the presence of foreign pollen may have several explanations. First, *L. salicaria* pollen may prevent *L. alatum*

pollen from adhering to stigmatic papillae (Galen and Gregory 1989; Randall and Hilu 1990). This could affect both morphs of *L. alatum* given the variety of sizes of *L. salicaria* pollen and the papillae lengths of *L. alatum* morphs (Mulcahy and Caporello 1970; and B. Brown, personal observation). Different papillae heights between morphs could accommodate different sizes of pollen so that some morph:donor combinations might be less or more effective than others in reducing seed set.

Second, *L. salicaria* pollen might usurp ovules by fertilizing them before *L. alatum* pollen. We think this is unlikely for two reasons. First, foreign pollination produces too few hybrids to account for the reduction in seed set for the mixed treatment compared to the legitimate treatment. Second, there are too few *L. salicaria* tubes at the base of the style to allow other factors such as fertilization and subsequent abortion of ovules to play a role in seed set reduction.

Third, *L. salicaria* pollen may prevent pollen germination or pollen tube growth, either mechanically (Thomson 1989) or chemically (Char 1977; Sukhada and Jayachandra 1980; Cruzan 1990; Murphy and Aarssen 1995a, b, c, d). It is unlikely that the reduction is due to pollen allelopathy given that previous studies of allelopathy show a considerable reduction in seed set with only a small number of allelopathic pollen grains present. For example, Thomson et al. (1981) found almost complete reproductive failure in ovule development with additions of 50% allelopathic pollen. Murphy and Aarssen (1995d) found a 70% reduction in seed set when pollen extract from only five pollen grains from *Phleum pratense* was applied to stigmas of *Agropyron repens*. Our data do not indicate such a dramatic decrease in seed set, and instead exhibit a more gradual decline with abundance of foreign pollen, suggestive of stigma or stylar clogging (Palmer et al. 1989; Scribailo and Barrett 1994) or stigma inhibition. Visual inspection of *L. salicaria* pollen on *L. alatum* stigmas 24 h after application (unpublished data) revealed corkscrew-shaped pollen tubes and oblate tips characteristic of an inhibition reaction at the stigmatic surface (Williams et al. 1982). Thus, events at the stigmatic surface are probably responsible for at least part of the reduction in seed set for mixed pollination. In experimental applications of mixed pollen where both donors were known, Cruzan (1990) found that certain donors appeared to have a “bad apple” effect, reducing pollen tube growth for any pollen which was applied simultaneously, although this is not always the case (Snow and Spira 1996). It is unlikely that this reduction in seed set in *L. alatum* is due to pollen limitation, since we applied excess conspecific legitimate pollen. A “bad apple” reaction or other stigmatic interference seem likely explanations for the partial reduction in seed set we observe in *L. alatum* with the presence of *L. salicaria* pollen.

The non-significant tendency towards greater seed set in the long morph for our legitimate and mixed treatments is typical of heterostylous species (Ganders 1979) but the reverse of this common pattern in the foreign

pollen treatment is noteworthy. We suggest that stylar inhibition may be responsible for this since *L. salicaria* tubes must travel farther through long morph styles (Thomson 1989), potentially allowing selective inhibition (Cruzan 1990, 1993) of foreign pollen, and causing reduced seed set in the long morph. Thus stylar inhibition of foreign pollen tubes may help reduce rates of hybridization. Such a reaction would also explain the less dramatic reduction in seed set with increasing presence of *L. salicaria* pollen.

#### Potential for hybridization and introgression

Although seed set with the foreign pollen treatment was extremely low, some putative hybrid seed formed (Fig. 1). Attempts to ascertain if these seeds were hybrids were unsuccessful due to their poor germination rate and very limited growth. Anderson and Ascher (1994) have found individuals with morphological characters intermediate between these two species in the field. They also report fertile crosses of *L. salicaria* and *L. alatum* (Anderson and Ascher (1993, 1994) in the laboratory. The work of Strefeler et al. (1996) with allozymes suggests that introgression may have already occurred between *L. salicaria* and *L. alatum*. If hybridization and/or introgression occur, the potential exists for the creation of a variety of *L. salicaria* which can invade the drier habitats characteristic of *L. alatum*. This could lead to invasion of even greater areas as previously limiting factors are overcome by importing adaptive capabilities from one species to another.

#### Potential for impact of foreign pollen in the field

We found that dye and – by inference – pollinators and pollen frequently move from *L. salicaria* to *L. alatum* separated by 1 m., a distance that frequently occurs in field populations. Thus, the potential for *L. salicaria* to decrease *L. alatum* seed set and reproduction, and perhaps even to hybridize with that species, may be an important threat to the persistence of *L. alatum* as a species.

#### Summary

Our research shows that flowering invasive species may pose a threat to native species by reducing seed set through stigma or stylar clogging. Furthermore, the potential for hybrid formation may exist for invasives which are congeneric with native species. Should such hybridization allow increased competitive ability for the invasive, native populations would be at even greater risk. Such reproductive and genetic effects, while challenging to detect, are potentially important to the survival of native species and warrant further research.

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#### References

- Anderson NO, Ascher PD (1993) Male and female fertility of loosestrife (*Lythrum*) cultivars. *J Soc Hortic Sci* 118:851–858
- Anderson NO, Ascher PD (1994) Erosion of style/anther length integrity in introgressive *Lythrum* hybrids. In: Stephenson AG, Kao T (eds) Pollen-pistil interaction and pollen tube growth. American Society of Plant Physiologists, Rockville, Md., pp 268–271
- Arroyo J, Dafni A (1993) Interspecific pollen transfer among co-occurring heteromorphic and homomorphic species. *Isr J Bot* 41:225–232
- Barrett SCH (1992) Heterostylous genetic polymorphisms: Model systems for evolutionary analysis. In: Barrett SCH (ed.), Evolution and function of heterostyly. Springer, Berlin Heidelberg New York, pp 1–29
- Bartsch D, Lehnen M, Clegg J, Pohl-Orf M, Schuphan I, Ellstrand NC (1999) Impact of gene flow from cultivated beet on genetic diversity of wild sea beet populations. *Mol Ecol* 8:1733–1741
- Bergman P (1996) Early flowers of *Bartsia alpina* (Scrophulariaceae) and the visitation of bumblebees. *Acta Bot Neerl* 45: 355–366
- Bing DJ, Downey RK, Rakow GFW (1996) Assessment of transgene escape from *Brassica rapa* (*B. campestris*) into *B. nigra* or *Sinapis arvensis*. *Plant Breed* 115:1–4
- Brown BJ (1999) The impact of an invasive species (*Lythrum salicaria*) on pollination and reproduction of a native species (*Lythrum alatum*). PhD thesis, Kent State University, Kent, Ohio
- Brown J, Brown AP (1996) Gene transfer between canola (*Brassica napus* L. and *B. campestris* L.) and related weed species. *Ann Appl Biol* 129:513–522
- Campbell DR, Motten AF (1985) The mechanism of competition for pollination between two forest herbs. *Ecology* 66:554–563
- Char MBS (1977) Pollen allelopathy. *Naturwissenschaften* 64: 489–490
- Cruzan MB (1990) Pollen-pollen and pollen-style interactions during pollen tube growth in *Erythronium grandiflorum* (Liliaceae). *Am J Bot* 77:116–122
- Cruzan MB (1993) Analysis of pollen-style interactions in *Petunia hybrida*; the determination of variance in male reproductive success. *Sex Plant Reprod* 6:275–281
- Cruzan, MB, Barrett, SCH. (1993) Contribution of cryptic incompatibility to the mating system of *Eichornia paniculata* (Pontederiaceae). *Evolution* 47:925–934
- Dafni A (1992) Pollination ecology: a practical approach. Oxford University Press, Oxford
- Darwin C (1877) The different forms of flowers on plants of the same species. Appleton, New York
- Ellstrand NC, Prentice HC, Hancock JF (1999) Gene flow and introgression from domesticated plants into their wild relatives. *Annu Rev Ecol Syst* 30:539–563
- Feinsinger P, Murray KG, Kinsman S, Busby WH (1986) Floral neighborhood and pollination success in four hummingbird-pollinated cloud forest plant species. *Ecology* 67:449–464
- Free JB (1970) The flower constancy of bumblebees. *J Anim Ecol* 39:395–402
- 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
- Ganders FR (1979) The biology of heterostyly. *N Z J Bot* 17: 607–635

- Graham SA (1975) Taxonomy of the Lythraceae in the south-eastern United States. *SIDA Contrib Bot* 6:80–103
- Jennersten O, Kwak M (1991) Competition for bumblebee visitation between *Melampyrum pratense* and *Viscaria vulgaris* with healthy and *Ustilago*-infected flowers. *Oecologia* 86:88–98
- Jennersten O, Berg L, Lehman C (1988) Phenological differences in pollinator visitation, pollen deposition and seed set in the sticky catchfly, *Viscaria vulgaris*. *J Ecol* 76:1111–1132
- Kohn JR, Waser NM (1985) The effect of *Delphinium nelsonii* pollen on seed set in *Ipomopsis aggregata*, a competitor for hummingbird pollination. *Am J Bot* 72:1144–1148
- Kwak M, Jennersten O (1986) The significance of pollination time and frequency and of purity of pollen loads for seed set in *Rhinanthus angustifolius* (Scrophulariaceae) and *Viscaria vulgaris* (Caryophyllaceae). *Oecologia* 70:502–507
- Levin DA (1970) Assortative pollination in *Lythrum*. *Am J Bot* 57: 1–5
- 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
- McGuire AD, Armbruster SD (1991) An experimental test for reproductive interactions between two sequentially blooming *Saxifraga* species (Saxifragaceae). *Am J Bot* 78:214–219
- Mulcahy DL, Caporello D (1970) Pollen flow within a tristylous species: *Lythrum salicaria*. *Am J Bot* 57:1027–1030
- Murphy SD, Aarssen LD (1995a) Reduced seed set in *Elytrigia repens* caused by allelopathic pollen from *Phleum pratense*. *Can J Bot* 73:1417–1422
- Murphy SD, Aarssen LD (1995b) Allelopathic pollen extract from *Phleum pratense* L. (Poaceae) reduces seed set in sympatric species. *Int J Plant Sci* 156:435–444
- Murphy SD, Aarssen LD (1995c) Allelopathic pollen extract from *Phleum pratense* L. (Poaceae) reduces germination, in vitro, of pollen of sympatric species. *Int J Plant Sci* 156:425–434
- Murphy SD, Aarssen LD (1995d) In vitro allelopathic effects of pollen from three *Hieracium* species (Asteraceae) and pollen transfer to sympatric Fabaceae. *Am J Bot* 82:37–45
- O'Neil P (1922) Variation in male and female reproductive success among floral morphs in the tristylous plant *Lythrum salicaria* (Lythraceae). *Am J Bot* 79:1024–1030
- O'Neil P (1994) Genetic incompatibility and offspring quality in the tristylous plant *Lythrum salicaria* (Lythraceae). *Am J Bot* 81:76–84
- Palmer M, Travis J, Antonovics J. (1989) Temporal mechanisms influencing gender expression and pollen flow within a self-incompatible perennial, *Amiatium muscaetoxica* (Liliaceae). *Oecologia* 78:231–236
- Petanidou T, Den Nijs JCM, Oostermeijer JGB (1995) Pollination ecology and constraints on seeds of the rare perennial *Gentiana cruciata* L. in the Netherlands. *Acta Bot Neerl* 44:55–74
- Proctor MP, Yeo P, Lack A (1996) The natural history of pollination. Timber Press, Portland
- Randall JL, Hilu KW (1990) Interference through improper pollen transfer in mixed stands of *Impatiens capensis* and *I. pallida* (Balsaminaceae). *Am J Bot* 77:939–944
- Rathcke B (1983) Competition and facilitation among plants for pollination. In: Real L (ed) *Pollination biology*. Academic Press, New York, pp 305–329
- Scheffler JA, Dale PJ (1994) Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. *Transgenic Res* 3:263–278
- Schemske DW (1981) Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. *Ecology* 62:946–954
- Scribailo RW, Barrett SCH (1994) Effects of prior self-pollination on outcrossed seed set in tristylous *Pontederia sagittata* (Pontederiaceae). *Sex Plant Reprod* 7:273–281
- Shore J, Barrett SCH (1984) The effect of pollination intensity and incompatible pollen on seed set in *Turnera ulmifolia* (Turneraceae). *Can J Bot* 62:1298–1303
- Snow AA, Spira TP (1996) Pollen-tube competition and male fitness in *Hibiscus moscheutos*. *Evolution* 30:1866–1870
- Strefeler MS, Darms E, Becker RL, Katovich EJ (1996) Isozyme characterization of genetic diversity in Minnesota populations of purple loosestrife, *Lythrum salicaria* (Lythraceae). *Am J Bot* 83:265–273
- Sukhada K, Jayachandra S (1980) Pollen allelopathy – a new phenomenon. *New Phytol* 84:739–746
- Thomson JD (1989) Germination schedules of pollen grains: implications for pollen selection. *Evolution* 43:220–223
- Thomson JD, Andrews BJ, Plowright RC (1981) The effect of a foreign pollen on ovule development in *Diervilla lonicera* (Caprifoliaceae). *New Phytol* 90:777–783
- Thompson DQ, Stuckey RL, Thompson EB (1987) Spread, impact, and control of purple loosestrife (*Lythrum salicaria*) in North American Wetlands. Fish and Wildlife Research 2. Fish and Wildlife Service, U.S. Dept. of the Interior, Washington, D.C.
- Waser NM (1978) Competition for hummingbird 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*. Academic Press, New York, pp 277–293
- Waser NM (1988) Comparative pollen and dye transfer by pollinators of *Delphinium nelsonii*. *Funct Ecol* 2:41–48
- 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
- Wilcove DS, Rothstein D, Dubow J, Phillips A, Jones E (1998) Quantifying threats to imperiled species in the United States. *BioScience* 48:607–615
- Williams EG, Knox RB, Rouse JL (1982). Pollination sub-systems distinguished by pollen tube arrest after incompatible interspecific crosses in *Rhododendron* (Ericaceae). *J Cell Sci* 53:255–277
- Winer BJ (1971) *Statistical principles in experimental design*. McGraw-Hill, New York