The effect of invasive *Lythrum salicaria* pollen deposition on seed set in the native species *Decodon verticillatus*

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**Abstract:** Relatively little attention has been paid to pollinator-mediated interactions among invasive and native plants in spite of the fact that pollen transfer between species in invaded communities has been shown to occur. In this study, we investigated the impact of pollen deposition from the invasive plant species *Lythrum salicaria* on seed set in a native species that is a member of the same family, *Decodon verticillatus*. Whole plants were subjected to hand pollination by conspecific (*D. verticillatus* only) or mixed (a 1:1 mixture of *D. verticillatus* and *L. salicaria*) pollen to determine if the addition of a mixed pollen load interferes with the ability of *D. verticillatus* pollen to set seed. We found the mixed pollen treatment reduced *D. verticillatus* seed set by 33.3% relative to the conspecific pollen treatment. Our study demonstrates that invasive plants have the potential to negatively impact the reproductive success of a native species through pollinator-mediated interactions. We discuss the potential implications of our findings to the evolution and persistence of native plant populations in invaded communities.

**Key words:** competition, heterospecific pollen deposition, invasive plant species, pollination.

**Résumé :** Bien que l’on sache que le transfert de pollen s’effectue entre les espèces dans les communautés envahies, on a accordé peu d’attention aux interactions par les pollinisateurs entre les plantes indigènes et envahissantes. Les auteurs ont examiné l’impact de la déposition de pollen provenant de l’espèce de plante envahissante *Lythrum salicaria* sur la formation des graines chez une espèce indigène de la même famille, le *Decodon verticillatus*. Ils ont soumis des plantes entières à la pollinisation manuelle avec du pollen conspécifique (*D. verticillatus* seul) ou mélangé (1 : 1 du *D. verticillatus* et du *L. salicaria*) pour vérifier si l’addition d’une charge de pollens mélangés interfère avec la capacité du pollen du *D. verticillatus* à former des graines. Les auteurs ont constaté que le traitement avec des pollens mélangés réduit la mise à graine chez le *D. verticillatus* d’environ 33,3%, comparativement au pollen conspécifique. Ces études démontrent que les plantes envahissantes peuvent exercer des impacts négatifs sur le succès reproducteur d’une espèce indigène par des interactions via les pollinisateurs. Ils discutent les implications potentielles de ces constatations sur la persistance des populations de plantes indigènes dans les communautés envahies.

**Mots-clés :** compétition, déposition de pollen hétérosppécifique, espèces de plantes envahissantes, pollinisation.

**Introduction**

Insect-pollinated plants generally rely on the successful transfer of pollen among conspecifics to set seed. The presence of a novel flowering plant species in a community can alter local pollinator movement patterns (Lopezaraiza-Mikel et al. 2007) and, as such, has the potential to affect pollen transfer and native fitness (reviewed in Bjerknes et al. 2007; Morales and Traveset 2009). Yet, little attention has been paid to the mechanisms underlying these fitness effects. Specifically, few studies have directly examined the impact of mixed pollen deposition on native seed set.

When invasive and native plants compete for pollinators, there can be negative impacts on quality and (or) quantity of pollen receipt for natives (Waser 1983). In the case of reduced pollen quantity, highly attractive invasive plants can draw pollinators away from natives, resulting in reduced conspecific pollen transfer, lower seed set, and the potential for lower fitness (Chittka and Schürkens 2001). In cases of reduced pollen quality, invasive plants can act as a source of foreign pollen in a community and, where pollinators visit multiple species, can have negative impacts on native seed set through heterospecific pollen deposition (Morales and Traveset 2008; Matsumoto et al. 2010).

For heterospecific pollen deposition to occur among insect-pollinated species, certain criteria must be satisfied.
The two species must co-flower and share pollinators, and the pollen of both species must be placed on a similar part of a pollinator’s body or in such a way that pollen from both species can be transferred to the same stigma (Morales and Traveset 2008).

If heterospecific pollen is transferred, there are a variety of ways that it can interfere with seed production. Clogging can result from foreign pollen physically interfering with conspecific pollen tubes by blocking the native stigma or style (Galen and Gregory 1989). A second possibility is that heterospecific pollen chemically interferes with the germination of conspecific pollen tubes, receptivity of the stigma, or development of ovules, in a process known as pollen allelopathy (Murphy and Aarssen 1995). Finally, if heterospecific pollen successfully germinates on the stigma and fertilizes ovules, hybrid formation may result, potentially reducing the number of ovules available for conspecific pollination and, in the case of hybrid inferiority, reducing offspring fitness (Burgess et al. 2008).

Previous research demonstrates that invasive plants can integrate into native plant-pollinator networks (Mommert and Waser 2002; Vila et al. 2009) and that alien pollen grains tend to dominate native pollen transfer webs (Lopenzarai-Mikel et al. 2007). Additionally, researchers have found examples of pollinators switching frequently between native and invasive species (Grabas and Laverty 1999; Brown et al. 2002), making it likely that natives in invaded communities will experience heterospecific pollen transfer. Finally, since invasive species, by definition, are new arrivals to their communities, it is unlikely that natives will have evolved responses to any negative consequences of invasive pollen deposition, making them excellent systems to examine mechanisms and measure selection pressures (Jakobsson et al. 2009).

The few studies that have specifically investigated heterospecific pollen deposition in relation to invasive species have reported mixed effects of alien pollen on native seed set, ranging from negative to neutral. For example, work done on interactions between the invasive Taraxacum officinale and the native relative Taraxacum japonicum indicates that supplemental hand-pollination treatments of mixed invasive and native pollen result in reduced seed set relative to hand pollination with conspecific pollen only (Matsumoto et al. 2010). In contrast, controlled hand-pollination experiments found that the application of pollen from the invasive species Carpobrotus spp. did not significantly impact seed set of the two native plants species in the genus Cistus (Moragues and Traveset 2005). This result is echoed by experimental work done to determine the impact of pollination of the native species Glaucium flavum with pollen from the invasive Solanum elaeagnifolium. In this system, pollination with mixed invasive pollen also resulted in no reduction in native plant seed set (Tscheulin et al. 2009). These mixed results suggest that more study is needed into pollinator-mediated interactions among invasive and native plants and that the consequences of heterospecific pollen deposition could be species and context dependent.

In this study, we explore the potential impacts of pollinator sharing among the invasive wetland plant Lythrum salicaria L. (Lythraceae) and the native Decodon verticillatus (L. Elliot). These two species are an ideal choice for a study of the effects of heterospecific pollen deposition because both co-flower in invaded wetlands of Southeastern Ontario, share many floral traits, have previously been observed to share pollinators (V. King, unpublished data), and are in the same family (Lythraceae). Preliminary observations indicate that L. salicaria pollen could be transferred to D. verticillatus stigmas in invaded sites through pollinator sharing (V. King, unpublished data) with the potential for negative impacts on seed set. Yet, recent related work demonstrated that, in the presence of L. salicaria, pollen limitation in D. verticillatus was reduced, relative to uninvaded sites (Da Silva et al. in preparation). This suggests a facilitative, rather than competitive, interaction between the two species and hints that negative impacts of heterospecific pollen deposition on D. verticillatus seed set are minimal. However, similar work conducted between L. salicaria and another co-flowering native, Lythrum alatum, found that invasive pollen deposition does reduce seed set with that particular species through interference at the stigmatic and stylar level, suggesting that competition through heterospecific pollen deposition does exist within the genus (Brown and Mitchell 2001). These contrasting results present an interesting puzzle that motivated this study.

Here, our objective was to determine the impact, if any, of heterospecific pollen transfer from the invasive plant L. salicaria on seed set in the native Ontario wetland species, D. verticillatus. Because of the northward range expansion and habitat preferences of L. salicaria, D. verticillatus populations are particularly likely to come into contact with this invasive plant. Moreover, since they both co-flower and share recent evolutionary history, several floral traits, and pollinators, D. verticillatus is likely to receive significant amounts of L. salicaria pollen. Yet, we know next to nothing about the impact of foreign pollen transfer between these two species. Below we describe the execution and results of a hand-pollination experiment designed to determine the impacts of heterospecific pollen transfer from L. salicaria on seed set in D. verticillatus.

Methods

Study species

Lythrum salicaria, or purple loosestrife, is a highly invasive wetland plant that is found throughout North America (Thompson et al. 1987). It exhibits a number of traits that likely contribute to its invasiveness, including lengthy and showy floral displays, large quantities of nectar, and ability to be successfully pollinated by a wide variety of pollinator taxa (Levin and Anderson 1970; O’Neil 1997; Comba et al. 1999). Lythrum salicaria predominantly reproduces sexually (Eckert 2001) and therefore relies on local pollinator networks for pollen transfer. Since its introduction to North America from Eurasia in the early 19th century, L. salicaria has attracted considerable attention from wetland ecologists and conservation biologists owing to its ability to rapidly invade and establish monotypic stands in wetland communities (Thompson et al. 1987; Brown et al. 2002). Its invasion of natural and seminatural systems provides a unique opportunity to study the effects of an alien invasive in a variety of communities and pollination contexts.

Decodon verticillatus, or swamp loosestrife, is a native...
North American member of the Lythraceae and, as such, shares many floral characteristics with *L. salicaria*, including similarities in floral shape, color, inflorescence architecture, and the presence of tristyley or three distinct style morphs (Graham et al. 2005). *Decodon verticillatus* can reproduce both sexually (via pollinator-mediated pollen transfer) or clonally (via aerenchymous tissue and adventitious roots). While *L. salicaria* is largely self-incompatible (but leaky in midstyled morphs (O’Neil 1994; Mal et al. 1999)), *D. verticillatus* is self-compatible in the northern edge of its range and thus is able to reproduce via pollinator movement within the same clone (Eckert 2002). However, inbreeding is costly because of the presence of elevated levels of inbreeding depression (Eckert and Barrett 1994). *Decodon verticillatus* is naturally found in wetland habitats throughout eastern North America, and owing to recent invasions, *L. salicaria* and *D. verticillatus* now co-occur at a number of locations.

**Pollen addition experiment**

Seeds from six *D. verticillatus* populations (three *L. salicaria* invaded, three uninvaded) were collected from the Queen’s University Biological Station (44.567°N, 76.324°W) and the surrounding area in September and October 2008. Seeds were cold stratified for 3 weeks at 4 °C and then transferred to trays for germination. Once the majority of seedlings had reached the four-leaf stage, they were transplanted to Pro-Mix soil in 10.2 cm pots and grown to flower in a glasshouse at the University of Ottawa under controlled temperature and light (temperature ranged from 22 °C to 30 °C and 16 h of light per d was maintained by artificial lighting). Plants were watered daily and fertilized biweekly with 20-20-20 fertilizer diluted to 2 g/L. Fifty-three *D. verticillatus* plants reached flowering, and of these, 13 were randomly assigned as pollen donors. The remaining 40 were randomly assigned to one of two groups: the conspecific-only (only *D. verticillatus*) pollen addition control group or the mixed (*D. verticillatus* and *L. salicaria*) pollen addition treatment group. Plants in the conspecific-only pollen control group were hand pollinated with *D. verticillatus* pollen collected from at least four donor plants (depending on flowering rates of the donor plants) and from both whorls of anthers from each flower. Plants in the mixed pollen treatment group were hand pollinated with an approximate 1:1 ratio of *L. salicaria* : *D. verticillatus* pollen grains (corresponding to an 8:1 ratio of anthers, to adjust for differences in pollen grain production between the two species). This method ensured that plants in the mixed treatment group received enough pollen to achieve maximum seed set, such that the only difference between pollination treatments is the presence or absence of *L. salicaria* pollen.

Each morning during the course of the experiment, anthers were collected from donor plants of both species and placed into two 1.5 mL microcentrifuge tubes, one for each treatment. The tubes were placed under a 60 W incandescent bulb for approximately 20 min to promote the full dehiscence of pollen. Following this, a toothpick was used to gently apply pollen from the tube to every receptive stigma on each treatment plant. The number of flowers pollinated on each plant was subsequently recorded. Treatments were applied every day for 4 weeks from the observation of the first flower on each plant, with each flower receiving multiple pollinations to more closely mimic natural pollination. On the last day of treatment, any unopened buds were tagged to mark where treatments ended, and plants were then left to set seed in the greenhouse for 2 weeks. After this point, all treated fruits were harvested and placed in coin envelopes, and their position on the inflorescence was recorded.

Seeds from first 30 fruits from each plant were hand counted and then averaged to produce a single value of average seed set for each plant. Owing to the close proximity of two of the uninvaded sites, plants sourced from these sites were grouped together and analyzed as one site, changing the number of sites from six to five. A partly nested analysis of variance (ANOVA) was used to determine if seed set differed across pollination treatments, taking into account variation by site. Invasion status of the site from which the seed was obtained was included in the model to investigate the potential for a response to treatment to differ based on the presence of the invasive plant *L. salicaria* in the source population. Assumptions for the partly nested split-plot model must be tested separately for between and within assumptions (Quinn and Keough 2002). Assumptions were met for normality and homoscedasticity (Shapiro–Wilk goodness-of-fit, *p* = 0.8053; Levene, *p* = 0.2946). Statistical analyses were performed on S-PLUS 8.0 (Insightful Corp., Palo Alto, California) or JMP 8.0.2 (SAS Institute Inc., Cary, North Carolina).

**Results**

Three *D. verticillatus* plants died prior to or shortly after flowering and were excluded from the study. Final sample sizes were 16 pure and 21 mixed treatment plants. Since our measure of seed set involved an average of the first 30 fruits and some plants produced less than this, we first conducted a *t* test on fruit production across treatments to ensure that any variance in fruit production did not correspond to treatments. Fruit set data was log-transformed to meet assumptions, and a *t* test revealed no significant differences in mean fruit set by treatment (*t* = −0.286, df = 35.0, *p* = 0.777). A partly nested split-plot analysis of seed set revealed significant differences by treatment, indicating that seed set was lower in the mixed pollen addition treatment than in the conspecific pollen addition control plants (Fig. 1). No other factors, including the interaction, were significant, indicating that the effect of the treatment did not vary according to the invasion status of the source population (Table 1).

Additional data collected on date of first flowering was analyzed using a nonparametric Kruskal–Wallis rank sum test. Differences in first flowering time were not significantly different when modeled by site alone (Kruskal–Wallis chi-square = 10.14, df = 5, *p* = 0.0713), and no effect of site or invasion status were detected using a nested model (*F* = 0.5991, df = 4, *p* = 0.6659; *F* = 1.4100, df = 1, *p* = 0.2435, respectively). Analysis of germination data revealed no significant difference in germination rates by site or invasion status (*F* = 1.7290, df = 4, *p* = 0.1570; *F* = 0.3736, df = 1, *p* = 0.5436, respectively).
Fig. 1. Least mean squares of Decodon verticillatus seed set (seeds per fruit) ± 2 SE in mixed invasive–native pollen and pure native pollen hand supplementations.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>F</th>
<th>P</th>
</tr>
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<tr>
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<td>136.391</td>
<td>0.7889</td>
<td>0.4416</td>
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<tr>
<td>Treatment</td>
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<td>853.559</td>
<td>11.6799</td>
<td>0.0465</td>
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<td>Invasion × treatment</td>
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<td>2.8211</td>
<td>0.1980</td>
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<tr>
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<td>494.077</td>
<td>2.3202</td>
<td>0.2537</td>
</tr>
<tr>
<td>Treatment × site</td>
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<td>212.949</td>
<td>1.8308</td>
<td>0.1654</td>
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<tr>
<td>Error</td>
<td>27</td>
<td>1046.8532</td>
<td>0.065</td>
<td>residual</td>
</tr>
</tbody>
</table>

Note: Site(invasion) was modeled as a random factor. All other factors were modeled as fixed.

*Tested over 1.0651 × Site(invasion) — 0.065 × residual.

**Tested over 1.0651 × treatment × site(invasion) — 0.065 × residual.

Denotes significance at the 0.05 level.

**Tested over treatment × site(invasion).

Discussion

Using pollen addition experiments conducted in a greenhouse, we found that seed set of the native plant D. verticillatus was lower when hand pollinated with a mixture of conspecific and invasive pollen than when pollinated with conspecific pollen alone. This demonstrates that the application of L. salicaria pollen interferes with the ability of D. verticillatus pollen to set a full complement of seed. If the negative effects of heterospecific pollen deposition observed here are mirrored in the field, our results could have important consequences for fitness and selection in invaded populations of D. verticillatus.

Our findings are consistent with those of Brown and Mitchell (2001), who reported that pollen applications of conspecific pollen mixed with pollen from the invasive plant L. salicaria resulted in 28.8% lower seed set than conspecific-only pollen applications in the native L. alatum, and that a pure invasive pollen addition resulted in very low seed set. However, they applied their treatments to individual flowers rather than whole plants, introducing the potential for resource reallocation within plants, which could inflate differences between treatments (Knight et al. 2005). The whole plant treatments employed in our study account for this by assigning all flowers on one plant to a single treatment. Still, we find results similar to those found by Brown and Mitchell (2001). In our study, mixed pollen resulted in a 33.3% reduction in seed set in D. verticillatus. This result suggests that pollinator sharing with L. salicaria could be detrimental for D. verticillatus since simultaneous transfer of both species’ pollen (i.e., a pollinator arrives on D. verticillatus carrying both species of pollen) results in decreased seed set. The mechanisms behind this decrease in seed set could lie in mechanical interference (stigma or stylar clogging) or chemical interference (Morales and Traveset 2008). Determining the mechanism(s) responsible for reduced mixed seed set will require further experimentation.

Our findings have interesting implications when considered along with previous work done in this system. Our study yields similar results to Brown and Mitchell’s (2001) greenhouse study of L. salicaria and L. alatum, suggesting that L. salicaria has the potential to reduce fitness in both co-flowering relatives. Yet, the similarities apparently do not extend to pollen transfer in situ. A related field study with D. verticillatus found that L. salicaria invaded sites suffer from less pollen limitation than uninvaded sites, suggesting a lack of negative impact of invasive pollen deposition on fitness on natives (Da Silva et al., in preparation). At the same time, field array experiments conducted with L. alatum support the finding of a negative impact of the invasive species on seed set (Brown et al. 2002). Uncovering the difference between the negative impacts of hand supplemented heterospecific pollen deposition experiments and tests with pollinator-mediated pollen transfer in situ will require more in-depth study.

A comparison of our study to others conducted with native species outside of Lythraceae reveals that our research adds more evidence for negative, rather than neutral, effects of invasive pollen deposition (Moragues and Traveset 2005; Tscheulin et al. 2009; Matsumoto et al. 2010). Some of the inconsistency in existing studies in this field may have to do with the influence of relatedness on the impact of invasive pollen deposition. A study by Moragues and Traveset (2005), for example, suggests that a potential explanation for a lack of a negative impact of invasive pollen application could be that the species are not close relatives, making pollen germination interference unlikely. This theory is echoed in a meta-analysis that concluded that the effect of aliens on reproductive success was most detrimental for natives when the two species shared similar floral symmetry or colour, suggesting the potential for the influence of phylogenetic relatedness on the effect (Morales and Traveset 2009). Since the natives L. alatum and D. verticillatus are part of the same family as their invader (Lythraceae), heterospecific pollen transfer may be more likely to be detrimental to their seed set. Interestingly, the other studies we found that conducted mixed invasive hand pollinations on native plants, those that had negative impacts were conducted using close relatives (Matsumoto et al. 2010) and those that found neutral effects were conducted using co-flowering nonrelatives...
(Moragues and Traveset 2005; Tscheulin et al. 2009), further supporting the notion that large differences in relatedness may influence the impact of supplemental invasive hand pollinations. Another possibility for the range of effects observed is the timing of invasive pollen deposition. In their study involving two native co-flowering species, Caruso and Alfaro (2000) found that the deposition of *Castilleja linariaefolia* pollen has a competitive effect on *Ipomopsis aggregata* flowers, but only when it is deposited first (rather than simultaneous deposition of both species’ pollen). To fully elucidate the impact of invasive pollen deposition on natives, more studies that examine a range of invasive and native species combinations and a variety of types and timings of pollen supplementation are needed.

If the deposition of heterospecific pollen on a native species reduces seed set consistently, selection should favour traits that mitigate these fitness losses. Examinations of stained stigmatic tissue under a microscope indicate that *L. salicaria* pollen is frequently present on *D. verticillatus* stigmas collected from the field (E. Da Silva, personal observation). This observation, combined with our findings, suggest that *D. verticillatus* is likely under selection to mitigate the negative effects of *L. salicaria* pollen on seed set. Changes in flowering time to reduce overlap in pollinator use, changes in anther position to alter pollen placement on pollinators, or even selection towards modes of reproduction that decrease reliance on pollinators (e.g., increased self-pollination) are all ways that plants can evolve to reduce heterospecific pollen deposition. While examples of this type of selection are abundant for co-flowering species (Waser 1978; Fishman and Wyatt 1999; Caruso 2000; Aizen and Vazquez 2006), studies on the selection of native phenotypes as a result of new species invasions are, to our knowledge, nonexistent. Interestingly, in a field experiment related to the present study, we have found evidence that the presence of *L. salicaria* in a community is also correlated with lower pollen limitation in *D. verticillatus* (Da Silva et al., in preparation). Taken together, our two studies indicate that the presence of *L. salicaria* can have multiple, potentially conflicting, influences on *D. verticillatus*’ reproductive success. While we are still in the process of investigating how these influences might operate to give an overall picture of the influence of *L. salicaria* on *D. verticillatus*’ seed fitness, we speculate that in the field, there is some benefit associated with the presence of the invasive species, such as higher pollinator visitation, that potentially outweighs the negative impacts, including the interference at the stigmatic level that we have described here.

We included the invasion status of the source population in our statistical model as we expected that we might observe evolved differences in the response of *D. verticillatus* plants sourced from communities that had encountered heterospecific pollen deposition when compared with those that had not. For example, if selection in invaded sites had favoured plants that were better suited to contend with heterospecific pollen, we expected that we might see evidence of increased seed set in the mixed treatment of seeds sourced from invaded sites relative to seeds sourced from uninvaded sites. Instead, our analysis of seed set found no significant differences by invasion status (Table 1). This result is not entirely surprising. First, there could be a lack of sufficient genetic variation in invaded sites to allow for selection on traits related to heterospecific pollen deposition. A second possibility is that the genetic variation in invaded sites is sufficient, but not enough time has passed since invasion for a response to be observable. A third possibility is that selection for heterospecific pollen deposition related traits exists in both invaded and uninvaded sites, making differences in seed set by site type unobservable.

Our research demonstrates that the deposition of heterospecific and invasive *L. salicaria* pollen on the native plant *D. verticillatus* is detrimental, has negative consequences for reproduction, and has potential implications for selection and evolutionary responses to invasion. Our study finds that the pollinator-mediated transfer of *L. salicaria* pollen has negative impacts on *D. verticillatus* fitness. This research adds to an increasing amount of evidence for an impact of invasive plants on pollinator-mediated reproductive success in natives, and raises interesting questions about the potential for natives to evolve ways to mitigate these threats.

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