PLANT-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Evidence for pollen limitation of a native plant in invaded communities

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Abstract Animal-pollinated invasive species have frequently been demonstrated to outcompete native species for pollinator attention, which can have detrimental effects on the reproductive success and population dynamics of native species. Many animal-pollinated invasive species exhibit showy flowers and provide substantial rewards, allowing them to act as pollinator 'magnets', which, at a large scale, can attract more pollinators to an area, but, at a smaller scale, may reduce compatible pollen flow to local native species, possibly explaining why most studies detect competition. By performing pollen limitation experiments of populations in both invaded and uninvaded sites, we demonstrate that the invasive plant *Lythrum salicaria* appears to facilitate, rather than hinder, the reproductive success of native confamilial *Decodon verticillatus*, even at a small scale, in a wetland habitat in southeastern Ontario. We found no evidence for a magnet species effect on pollinator attraction to invaded sites. Germination experiments confirmed that seeds from invaded sites had similar germination rates to those from uninvaded sites, making it unlikely that a difference in inbreeding was masking

competitive effects. We describe several explanations for our findings. Notably, there were no differences in seed set among populations at invaded and uninvaded sites. Our results underscore the inherent complexity of studying the ecological impacts of invasive species on natives.

Introduction

The introduction of a novel plant species to a community can result in new ecological interactions with native plants in the community. These can range from competitive, in which the introduced species and native species vie for limited resources (D'Antonio and Mahall 1991; Hamilton et al. 1999; Ridenour and Callaway 2001), to facilitative, where mutually positive interactions aid in the establishment of the introduced species (Laverty 1992; Rodriguez 2006). In insect-pollinated flowering plant communities, these interactions can extend to competition for, and/or sharing of, pollinator services (Bjerknes et al. 2007; Moeller 2004; Munoz and Cavieres 2008; Traveset and Richardson 2006; Vila et al. 2009).

An introduced plant species can negatively impact pollinator-mediated fitness of native species via several avenues. First, by acting as a foreign pollen source in the community, an introduced plant may contribute to heterospecific pollen transfer, leading to a decrease in the quality of pollen received (Jakobsson et al. 2008; Lopezaraiza-Mikel et al. 2007). Secondly, by usurping pollinators that may have otherwise visited native species, an introduced plant can reduce the quantity of pollen received (Waser 1983). A reduction in pollen quantity and/or quality is

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often associated with pollen limitation, defined as inadequate receipt of compatible pollen grains resulting in reduced seed and/or fruit production (Burd 1994; Knight et al. 2005). A recent review reported that 62 % of the 258 species surveyed exhibited pollen limitation (Ashman et al. 2004; Burd 1994). Finally, the presence of a novel plant species in a community can lead to changes in the probability of self-pollination (Yang et al. 2011), which may in turn impact seed fitness through inbreeding depression.

The presence of an invasive plant species in a community can reduce pollinator visitation rates to natives, leading to reduced seed and/or fruit set (Brown and Mitchell 2001; Chittka and Schurkens 2001; Grabas and Laverty 1999; Totland et al. 2006). Furthermore, the loss of reproductive opportunity due to the presence of a competitive co-flowering species has been shown to alter patterns of selection on native floral traits (Caruso 2000; Smith and Rausher 2008). Bjerknes et al. (2007), in a review of the seven studies available at the time, found that all the studied systems were dominated by competitive interactions.

A smaller body of research indicates that co-flowering species can facilitate pollinator-mediated reproduction. Moeller (2004) demonstrated that populations consisting of co-occurring congeners of *Clarkia* had higher pollinator availability and lower pollen limitation when compared to populations consisting of single species. Similarly, Molina-Montenegro et al. (2008) found that, after accounting for density, the invasive flowering shrub *Lupinus arboerus* acted as a "magnet species" for the less attractive invasive *Cardus pycnocephalus*, increasing pollinator visitation rates and seed output where the two species grow together. If a highly attractive invasive species acts as a magnet species for native plants and facilitates pollination by drawing more pollinators to a patch, we expect native species to suffer less pollen limitation in invaded than uninvaded communities (Thomson 1978), although the possibility remains that the increased visitation could result in higher selfing rates (Yang et al. 2011), a consequence of mixed species communities that has received almost no attention.

In addition to its impacts on the plant community, the direction of the interaction between an invasive and the native plant community has implications for the size and persistence of local pollinator populations (Bjerknes et al. 2007; Williams et al. 2011). The introduction of a novel plant species to a community has generally been shown to increase the density of local pollinator populations (Westphal et al. 2003; but see also Williams et al. 2011). Furthermore, the presence of an introduced plant resource may help to extend the geographical or seasonal limits of interacting insects (Graves and Shapiro 2003; Jahner et al. 2011). It has also been suggested that the presence of

invasive species could enhance pollination services by native pollinators in wild and agricultural settings (Levy 2011), although, in some habitats, alien plants may indirectly contribute to the loss of pollinator abundance and diversity (Holzschuh et al. 2011).

To our knowledge, no previous study has explicitly compared pollen limitation and the resulting seed fitness of a native plant in naturally occurring invaded and uninvaded communities. Previous studies have examined how the presence of an invasive can lead to changes in pollinator visitation patterns (Lopezaraiza-Mikel et al. 2007), and/or plant seed set (Chittka and Schurkens 2001), but the role of pollination in these in these effects must be interpreted carefully (Bjerknes et al. 2007). While studies of differences in seed set among invaded and uninvaded populations can give important insight into the impacts of an invasive on plant fitness, it is not possible to attribute a change in seed set to pollinator-mediated interactions unless pollen supplementation is performed. Similarly, differences in visitation do not necessarily translate into differences in seed set.

In this study, our goal was to determine if the presence of the invasive species *Lythrum salicaria* L. (Lythraceae) alters pollinator-mediated reproductive success when it co-occurs with the native plant *Decodon verticillatus* (L. Elliot) (Lythraceae). Since these two species share recent evolutionary history (both are in Lythraceae), rely on a similar pollinator community (King and Sargent 2012), and co-flower for approximately 3 weeks in late summer, they provide a highly suitable study system in which to test the impact of an invasive species on pollinator-mediated reproductive success in a native.

Materials and methods

Study species and sites

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 common to alien invasive plant species, including lengthy and showy floral displays, large quantities of nectar, and generalist pollinator requirements (Comba et al. 1999; Levin 1970; O'Neil 1997). *Lythrum salicaria* predominantly reproduces sexually (Eckert 2002a), and therefore relies on local pollinator networks for pollen transfer. Since its introduction to North America from Eurasia in the early nineteenth century, *L. salicaria* has attracted much attention from wetland ecologists and conservation biologists due to its ability to rapidly invade and establish monotypic stands in wetland communities (Brown et al. 2002; Thompson et al. 1987). Its invasion of natural and

semi-natural 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 shares many floral characteristics with *L. salicaria*, including similarities in floral shape, color, inflorescence architecture, and the presence of tristyly (Graham et al. 2005). *Decodon verticillatus* can reproduce both sexually (via pollinator-mediated pollen transfer) and clonally (via arenchymous tissue and adventitious roots). While *L. salicaria* is largely self-incompatible (but leaky in midstyled morphs; Mal et al. 1999; O'Neil 1994), *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 2002b). However, inbreeding may be costly due to inbreeding depression (Eckert and Barrett 1994). In greenhouse experiments, *D. verticillatus* plants resulting from selfed seed produced fewer seeds with low/ later germination rates, and were smaller in stature (Eckert and Barrett 1994). Selfing rates in populations of southeastern Ontario have been shown to be around 30 %, and estimates of inbreeding coefficients range from 0.49 to 1.79 (Eckert 2002b; Eckert and Barrett 1994). *Decodon verticillatus* is found naturally in wetland habitats throughout eastern North America, and, due to recent invasions, *L. salicaria* and *D. verticillatus* now co-occur at a number of locations.

To determine how the presence of *L. salicaria* influences reproduction in *D. verticillatus*, a field study was conducted at the Queen's University Biological Station on Lake Opinicon, Ontario (44.567°N, 76.324°W) during the summer of 2009. Naturally occurring populations of *L. salicaria* and *D. verticillatus* were surveyed in June 2009, and from these, five sites containing *D. verticillatus* were chosen for study based on similarities in plant density and habitat type. Three sites were categorized as "invaded" (containing both *L. salicaria* and *D. verticillatus)* and two were categorized as "uninvaded" (containing *D. verticillatus* only) (Table 1). Sites were a minimum of 200 m apart.

Pollinator observations

Pollinator observations were conducted in each of the five sites. Observations were conducted in a 6 × 3 m permanently cordoned-off plot that exhibited plant density and diversity that was representative of the site. Each observation period was conducted with three observers placed at designated locations around the outskirts of the plot, in order to have maximum coverage. The flowering densities of *D. verticillatus* and *L. salicaria* in the observation plot were recorded prior to each observation period as the number of flowering branches or inflorescences (Table 1). Observations were conducted for 30 min twice a week from July 30 through August 25. Each site was observed for a minimum of five and a maximum of eight periods. Following Totland et al. (2006), each observer monitored a section of the plot and recorded the number of pollinators entering the plot and then visiting *D. verticillatus* in their section. With very few exceptions, observers reported that they were able to record all pollinators entering and visiting plants in their section. Pollinators were identified visually as they foraged and categorized according to the following functional groups: *Bombus*, Lepidopteran, *Apis mellifera*, solitary bee, Syrphid fly or wasp.

Pollen limitation

To measure the effect of the presence of *L. salicaria* on the reproductive success of *D. verticillatus*, pollen limitation experiments were conducted at each of the five sites. Prior to flowering, 30 branches of *D. verticillatus* at each site were haphazardly chosen and assigned to one of two treatments: open or supplemental pollination. To control for the potential relatedness of treatment branches (due to clonality), experimental branches were selected to be at least 2 m apart, which has previously been determined as the likely minimum distance between distinct clones in a population (Dorken and Eckert 2001). Additionally, to account for altered allocation between sexual and clonal reproduction in different branches, those chosen for the

Table 1 Variation among sites in number of pollinators observed entering a patch over a 30-min interval and *Decodon verticillatus* flowering descriptors

Site	Invasion status	Number of pollinators (30 min)	Flowering density	Morphs present (%)
Cow Island Marsh (CM)	Invaded	35.8 ± 7.6 (6)	17.7 ± 2.6 (6)	7.4 L:92.6 S
Deadlock Bay B (DBB)	Uninvaded	41.9 ± 6.0 (7)	23.8 ± 10.7 (7)	76.2 L:23.8 S
Indian Lake (IL)	Invaded	54.8 ± 6.1 (5)	43.3 ± 11.9 (5)	24.1 L:65.5 M:10.3 S
Little Island (LI)	Uninvaded	56 ± 14.1 (5)	24.8 ± 4.7 (5)	65.4 L:34.6 S
Silo Bay (SB)	Invaded	57.9 ± 8.3 (8)	21.5 ± 7.8 (8)	41.7 L:58.3 S

Values for columns 3 and 4 are presented as $\bar{x} \pm$ SE ($n =$ number of observation intervals). Flowering density measured as number of flowering branches within a $2 m^2$ plot. Morph ratios are presented as the percentage of all treatment branches at a site

L long-styled, *M* mid-styled, *S* short-styled morph

experiments were matched, as closely as possible, for the number of flower buds. To control for the potential impact of resource reallocation among flowers on the same branch, treatments were applied to whole branches rather than individual flowers on the same branch (a common limiting factor for pollen limitation tests in situ; Ashman et al. 2004). All branches were visited twice a week to record the number of open flowers and to track phenology. Open pollinated branches were left unmanipulated to assess seed set in *D. verticillatus* under natural levels of pollination. Supplemented branches were hand-pollinated with *D. verticillatus* pollen from five haphazardly chosen branches from the same population. Donor branches were chosen from at least 2 m away from any treatment plant to reduce the potential for self-fertilization by pollen collected from branches of the same clone, and a different set of five donor plants were chosen for each site visit. To supplement flowers, anthers were collected from both style morphs on donor flowers with a pair of forceps and placed in a tube. Once all donor anthers had been collected, pollen was mixed thoroughly and gently applied to all treatment plant stigmas with a toothpick. Supplemental pollination was performed on all open flowers on all supplemental treatment branches twice a week throughout the entire flowering period to mimic natural pollen deposition.

Once flowering was complete, *D. verticillatus* branches were left to set seed in the field. Fruits from treatment branches were collected prior to dehiscence in September 2009. Fruits were left to air-dry in the laboratory, and all fruits and seeds from each branch were counted. Seeds from each fruit were spread out on white paper and imaged with a digital camera (Canon EOS Rebel XSi). Images were then used to semi-automatically count seeds for each fruit using a custom-made program written in Matlab (The Mathworks, http://www.mathworks.com). Seeds per fruit were averaged for all fruits from the same branch to provide one mean seeds per fruit value for each branch.

Germination

In the fall of 2010, germination trials were conducted in a rooftop greenhouse at the University of Ottawa to compare the fitness of *D. verticillatus* progeny from invaded and uninvaded populations. Twenty seeds, from each of the approximately 15 open-pollinated branches collected at each site (described above), were randomly selected from fruits. Seeds were cold stratified for 2.5 weeks at 4 °C in moist diatomite. The seeds were subsequently treated with No-Damp[TM] fungicide and planted in trays containing Pro-Mix[TM] soil (75–85 % *Sphagnum* peat moss by volume, perlite, and limestone). The trays were placed in larger water-filled trays, and the soil surface was misted daily, to ensure high water levels during germination. Trays were rotated weekly. Greenhouse heating and cooling points during day and night were, respectively, set to 25/30 and 22/30 °C. Artificial lighting provided 16 h of light per day. Germination was recorded daily for 17 weeks as seeds continued to germinate throughout this period.

Statistical analysis

Our experimental design for the test of pollen limitation has three categorical factors of interest: invasion status, site, and treatment (open or supplemented). Since each site is scored as either "invaded" or "uninvaded", we analyzed our data using a partly nested split-plot design with site being nested within invasion status and each level of treatment (supplemented or open) being applied at each site. Pollinator visitation and germination data were analyzed using a nested model with invasion status and site nested within invasion status. Analyses for all data were performed on JMP 8.0.2 (SAS 2009).

Results

Pollinator observations

A nested ANOVA indicated no effect of invasion status on the total number of pollinators entering the observation area during the set time period (Table 2). Additionally, there was no interaction between the pollinator type (i.e., Syrphid flies, bumblebees, honeybees, butterflies, and solitary bees) and invasion status (Table 2), indicating that the pollinator community did not differ significantly among invaded and uninvaded sites. There was a significant effect of pollinator type on the number of pollinators entering the plot (Table 2), with bumblebees being significantly more common than other types of pollinators at all sites (Fig. 1).

Table 2 Results of a partly nested split-plot ANOVA comparing the type and number of pollinators visiting *D. verticillatus* during 30-min periods in observation plots at sites with or without *L. salicaria* invasion

Source	df	SS	F	P
Invasion	1	0.057	0.0012	0.973
Pollinator type	5	400.5	8.54	<0.001
Invasion × pollinator type	5	14.2	0.304	0.903
Site(Invasion)	3	21.3	0.454	0.719
Error	15	703.6		

Site(Invasion) was treated as a random factor; all factors were modeled as fixed
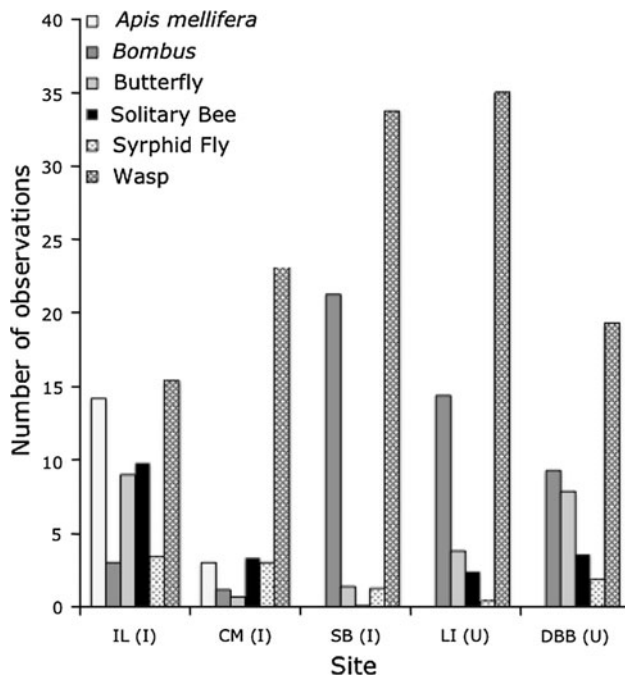
Fig. 1 Number and identity of pollinators recorded visiting *D. verticillatus* in each site during 30-min pollinator observation periods. Site codes are defined in the legend of Table 1. *Letters in parentheses* following each site name indicates whether the site was invaded (*I*) or uninvaded (*U*)

Pollen limitation

Of the 150 branches that were pre-assigned to the treatment category in total over the five sites, 23 were excluded due to herbivory, lack of flowering, rotten seed capsules, or otherwise uncountable seeds. Our analysis therefore includes all seeds from all fruits of the remaining 127 treatment branches. We found a significant effect of treatment on the mean number of seeds per fruit (Table 3), and this effect differed by invasion status with supplemented branches setting more seeds than open branches in the uninvaded populations, but not in invaded populations (Fig. 2; Table 3). No other model effects were significant (Table 3). We performed the identical analysis on fruit set (Table 3). There was no statistically significant effect of our treatment on fruit set, which is consistent with this being a more coarse measure of reproductive success than seed number. Accordingly, there was no significant interaction between the treatment and the invasion status of the site for fruit set. Interestingly, in contrast to seed set, we saw a trend towards a difference in fruit set between invaded and uninvaded sites (Table 3), with plants at invaded sites setting more fruit than those at uninvaded sites.

Germination

A nested GLM using an exponential distribution with a reciprocal link function revealed no significant difference

Table 3 Results of a partly nested split-plot ANOVA of seed and fruit set in *D. verticillatus* in sites with or without *L. salicaria* invasion

Source	df	SS	F	P
Seed set				
Invasion[a]	1	46.3	0.286	0.629
Treatment[b]	1	250.9	10.3	0.03
Invasion × treatment[b]	1	185.9	7.60	0.047
Site(Invasion)[c]	3	499.2	7.78	0.063
Treatment × site(Invasion)	3	64.2	0.256	0.857
Error	117	9,778.6		
Fruit set				
Invasion[d]	1	21,092.4	8.23	0.064
Treatment[e]	1	1,514.5	5.35	0.103
Invasion × treatment[e]	1	276.6	0.978	0.395
Site(Invasion)[f]	3	2,567.1	9.12	0.0512
Treatment × site(Invasion)	3	281.6	0.331	0.803
Error	127	107,893		

Site(Invasion) was treated as a random factor. All other factors were modeled as fixed

[a] Tested over $0.9505 \times$ Site(Invasion) $+ 0.0495 \times$ Residual

[b] Tested over 0.9505 Treatment × Site(Invasion) $+ 0.0495 \times$ Residual

[c] Tested over Treatment × Site(Invasion)

[d] Tested over $0.9977 \times$ Site(Invasion) $+ 0.0023 \times$ Residual

[e] Tested over $0.9977 \times$ Treatment × Site(Invasion) $+ 0.0023 \times$ Residual
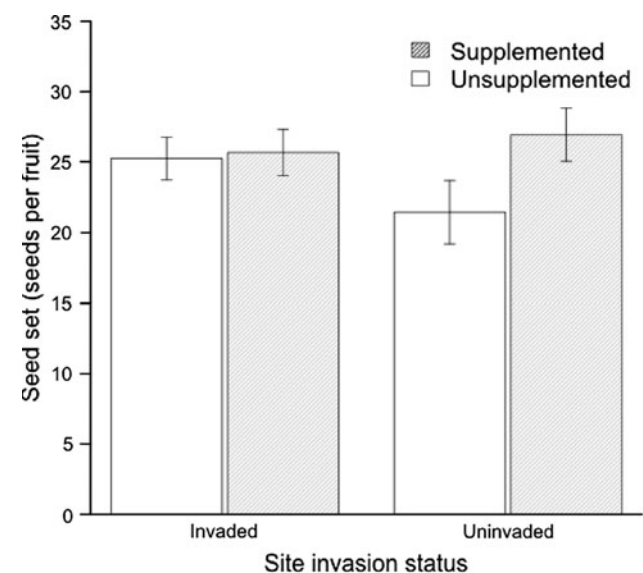
[f] Tested over Treatment × Site(Invasion)

Fig. 2 Least squares means of *D. verticillatus* seed set (seeds per fruit) for open and supplemented treatment branches in invaded and uninvaded communities. *Bar heights* represent least squares mean ± SE. Due to lack of survival of some treatment and control plants, final sample sizes were: $n_{invaded, \, unsupplemented} = 43$, $n_{invaded, \, supplemented} = 37$, $n_{uninvaded, \, unsupplemented} = 20$, $n_{uninvaded, \, supplemented} = 27$

in the number of germinating seeds emerging per day between invaded and uninvaded sites (LR $\chi^2 = 3.25$, $df = 1$, $p = 0.07$). On average, a slightly larger percentage of seeds germinated from plants in uninvaded sites ($\bar{x}_{\text{invaded}} = 10.4\%$, $\bar{x}_{\text{uninvaded}} = 12.4\%$) but the difference was not significant.

Discussion

We found that *D. verticillatus* populations at sites invaded by *L. salicaria* suffered less pollen limitation than populations at sites that were not invaded, suggesting that the presence of the invasive plant *L. salicaria* could facilitate pollinator-mediated fitness in the native plant *D. verticillatus*. Contrary to the predictions of the 'magnet species hypothesis' (Molina-Montenegro et al. 2008), we found no difference in the pollinator visitation rate to invaded and uninvaded populations. A recent review of other studies that collected visitation rate along with seed and/or fruit set revealed that the correlation between visitation rate and reproductive success is not always consistent; the explanations for the inconsistencies are diverse (Bjerknes et al. 2007).

To our knowledge, this is the first report of a native species having reduced pollen limitation in the presence of an invasive species. Most prior studies investigating the impacts of invasive plants have emphasized pollinator visitation (e.g., Chittka and Schurkens 2001; Lopezaraiza-Mikel et al. 2007), but not the resulting seed set. Pollen limitation experiments provide important information because they can reveal the difference between actual seed set and the seed set that would be achieved if pollinators were not limiting (Burd 1994). However, the results need to be carefully interpreted in order avoid conflating pollen limitation with resource limitation.

A recent meta-analysis of alien versus native impacts on co-flowering native plants found predominantly detrimental impacts of alien plants on pollinator visitation and seed set of natives, particularly when the alien and native have similar floral symmetry or color (Morales and Traveset 2009), echoing the conclusions of a recent review paper (Bjerknes et al. 2007). All previously published studies of the impact of *L. salicaria* on co-occurring natives have reported competitive, rather than facilitative, interactions (Brown et al. 2002; Grabas and Laverty 1999).

It is surprising that evidence for facilitative pollinator-mediated interactions between invasive and native plants is scarce, given that animal-pollinated invasives tend to be showy and attractive to pollinators (Rodriguez 2006). Moreover, the presence of alien and crop plants has been shown to increase pollinator abundance (Levy 2011; Westphal et al. 2003). So why is there no evidence for a concomitant increase in the pollination of native species?

We found only two published reports of an invasive plant facilitating reproduction (Munoz and Cavieres 2008; Nielsen et al. 2008). Studies demonstrating a so-called 'positive' impact of an invasion may be more difficult to publish, leading to a 'file drawer' effect, or this field of study may simply be under-represented.

We compared the germination of seeds from open-pollinated branches in invaded and uninvaded sites as an indirect indicator of inbreeding depression. Previous studies in *D. verticillatus* have revealed that inbred seeds exhibit lower germination (Eckert and Barrett 1994). We found no statistically significant difference in germination rate by invasion status, suggesting that the presence of the invasive species did not significantly alter selfing and inbreeding depression. Furthermore, there was no impact of invasion status on overall seed set in our populations (Table 3).

There are two likely mechanisms by which pollinator-mediated interactions among plant species can be facilitative (Moeller 2004). First, pollinators may respond numerically to the increase in plant density when multiple species occupy a site. Although we do not have exact sizes for *L. salicaria* populations in invaded sites, we do know that the *D. verticillatus* populations were of similar size across sites (Table 1). Thus, the invaded sites contained a larger and more diverse community of flowering plants, which may in turn lead to more pollinator attraction and higher visitation. However, we found that visitation rates did not differ among the two site types.

A second mechanism for facilitation described by Moeller (2004) is that the presence of multiple plant species, especially if their combined flowering times span a greater part of the year than either species would alone, contributes to the maintenance of a stable residential pollinator community. This explanation may have relevance for this system as the number of weeks that invaded communities contain blooms is greater than the number of weeks that uninvaded communities contain blooms (Da Silva, unpublished data). Furthermore, the pollinator community of *D. verticillatus* contains a number of species that may be vulnerable to gaps in resource availability, such as solitary bees (Roulston and Goodell 2011). More detailed examination of the pollinator community at our sites are being planned to explore this hypothesis.

We acknowledge that there could be other factors influencing pollen limitation in invaded sites. Haig and Westoby (1988), proposed that seed set is limited not only by adequate pollen receipt but also by the availability of resources to be provisioned to seed production. Our approach of pollinating entire branches rather than whole plants, while being the only logistically feasible option (due to submerged and/or attached branches), could have biased our results towards finding pollen limitation. Tests

of pollen limitation applied to anything other than entire plants may exaggerate differences between supplemental and open treatments, since plants may be able to shift resources from unsupplemented to supplemented flowers (Ashman et al. 2004). However, even if *D. verticillatus* plants were capable of shifting resources among branches, we have no reason to expect that the magnitude of this effect would differ between invaded and uninvaded sites.

Since we did not experimentally introduce *L. salicaria* into previously uninvaded populations, there could be site differences that correspond with invasion status such as differences in habitat availability, style morph ratios, soil nutrients, and shading. If, for example, invasion status and site resource status are correlated, and plants in resource-rich sites are more capable of maturing seed from the supplemental pollen, we could incorrectly attribute differences in sites to invasion that are actually related to resource availability. We attempted to add potted *D. verticillatus* to invaded and uninvaded sites; however, high mortality in the field stymied this effort at a manipulative experiment. Finally, we found no statistically significant effect of the interaction between morph ratio and invasion status on pollen limitation ($F = 1.63$, $df = 1$, $p = 0.2075$, tested seed set in supplemented branches removing M morphs since not represented across sites).

Our study adds a counterpoint to the pervasive view that invasive plants interact competitively for pollinator attention in native plant communities, and adds complexity to our understanding of the impact of invasive species on native ecosystems. Our findings also have implications for the potential for alien species to aid in the maintenance of existing pollinator communities.

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