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POLLINATOR-MEDIATED IMPACTS OF THE INVASIVE PLANT SPECIES *LYTHRUM SALICARIA* ON THE NATIVE *DECODON VERTICILLATUS*

ELIZABETH M. DA SILVA

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ABSTRACT

Plant-pollinator interactions in invaded communities can range from facilitative to competitive, with the latter being most commonly observed. If invasive species can successfully integrate into native plant-pollinator networks and change pollinator foraging patterns, native plant fitness in these communities could be altered.

Here I examine the impact of the showy flowering invasive plant, purple loosestrife (*Lythrum salicaria*), on a co-flowering close relative, swamp loosestrife (*Decodon verticillatus*). Both species are in the family Lythraceae, share a number of floral traits, and tend to be visited by the same pollinator community. A pollen limitation experiment was conducted in invaded and uninvaded populations of *D. verticillatus* on Lake Opinicon, Ontario, to determine if the presence of the *L. salicaria* altered pollinator driven reproductive success in *D. verticillatus*. These experiments demonstrated that invaded communities suffer less pollen limitation than uninvaded communities, suggesting that there could be a positive impact of invasion on this particular measure of plant fitness. Complementary pollinator observations conducted at the same sites determined that the facilitative effect was not due to increased pollinator visitation to *D. verticillatus*, but rather suggest that there are differences in pollinator foraging patterns on inflorescences in invaded and uninvaded sites. While greenhouse experiments revealed a negative impact of *L. salicaria* pollen deposition on seed set in *D. verticillatus*, our field experiments suggest that invasive pollen transfer to natives *in situ* is not great enough to significantly mitigate the facilitate effects of an invasion on seed set.

This work provides a unique contribution to the field of pollination biology as well as our understanding of the impacts of plant invasions, and suggests that positive as well as negative consequences for natives should be considered when investigating community impacts on plant invasion.

TABLE OF CONTENTS

Abstract ii

Table of Contents iv

List of Tables v

List of Figures vi

Acknowledgements vii

Chapter 1 – General Introduction and Overview 1

Statement of Thesis Contributions 2
Invasive plants and plant-pollinator interactions 2
Study system and research questions 4
References 7

Chapter 2 – Manuscript: The impact of an invasive plant species on pollinator-mediated fitness in a native: evidence for facilitation 9

Introduction 9
Methods 11
Results 15
Discussion 16
Conclusions 20
References 22

Chapter 3 – Manuscript: The effect of invasive pollen deposition on seed set in a native plant 28

Introduction 28
Methods 32
Results 35
Discussion 36
References 40

Chapter 4 – Discussion and Future Directions 45

LIST OF TABLES

Table 2.1 24
Details on D. verticillatus sites used in this study

Table 2.2 25
Results of a split-plot ANOVA of seed set in D. verticillatus in sites with or without L. salicaria invasion

Table 3.1 43
Results of a split-plot ANOVA of seed set in D. verticillatus in pure and mixed pollination treatments.

LIST OF FIGURES

Figure 2.1 26

Least mean squares ± 2 SE of D. verticillatus seed set (seeds per fruit) for open and supplemented treatment branches in invaded and uninvaded communities (open bars = open pollinated branches, shaded bars = supplementally pollinated branches).

Figure 3.1 44

Least mean squares of D. verticillatus seed set (seeds per fruit) ± 2 SE in mixed invasive-native pollen and pure native pollen hand supplementations

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Chapter 1:

GENERAL INTRODUCTION AND OVERVIEW

The introduction of a new plant species to a community can give rise to a variety of new ecological interactions. For plants that require a pollinator for pollen transfer, the presence of a novel flowering plant species in the community can disrupt pollinator movement and alter pollen transfer and seed set, with both of these having potential repercussions on fitness (reviewed in Morales and Traveset 2009 and Bjerknes et al. 2007). Invasive flowering plants provide a unique opportunity to study the alteration of pollination networks, as their successful establishment in many communities suggests that they possess traits that allow them to infiltrate local plant-pollinator networks.

For my thesis work, I investigated the potential for the invasive flowering plant, purple loosestrife (*Lythrum salicaria* L., Lythraceae), to alter pollinator-mediated reproduction in a co-flowering and frequently co-occuring native Ontario plant from the same family, swamp loosestrife (*Decodon verticillatus* L. Elliot, Lythraceae). In the following chapters, I report the results of pollen limitation experiments, pollinator observations and greenhouse experiments to examine the potential for *L. salicaria* introductions to alter pollinator-mediated reproduction and fitness in *D. verticillatus*. I begin this chapter by reviewing the literature and theory that motivated my research. I then briefly discuss the study system and introduce my hypotheses. In Chapter 2, I present the results of field observational and experimental studies. The results from greenhouse experiments are discussed in Chapter 3. Finally, Chapter 4 will explore the broad implications of my work, relate the results of the two experimental chapters to each other, and present new directions for research that this work has revealed.

STATEMENT OF THESIS CONTRIBUTIONS

The two chapters that follow this introduction are written as stand-alone manuscripts for submission to academic journals, with Chapter 2 having already been submitted for publication as a "Note" to *The American Naturalist.* As such, despite my attempts to reduce repetition, there remains a small amount of overlap in material between these two chapters, particularly in introductory background information and species descriptions. Additionally, the pronoun 'we' is used throughout Chapters 2 and 3, as these manuscripts have already been/will be submitted for publication with my supervisor and additional co-authors (Chapter 2 submitted with V. King and A-G Rolland-Lagan). However, excepting pollinator observation work (conducted in collaboration with V. King as USRA research) and the development of seed counting software (done by A-G. Rolland-Lagan) in Chapter 2, all research and writing was completed by myself, with the assistance of those mentioned in the Acknowledgements section.

INVASIVE PLANTS AND PLANT-POLLINATOR INTERACTIONS

Biological invasions present new challenges to existing ecosystems and have been purported to be a significant component of global environmental change, with plant invasions being of particular interest due to the tendency for higher numbers of invasive plants than animals (Vitousek et al., 1996).

Invasive plants that rely on animal pollination are an interesting sub-set of invasive plants since they are at the mercy of local pollinator networks to fulfill their reproductive requirements. Yet, they are often not only able to establish but also successfully compete with native flowering plants for pollinators (Traveset and Richardson 2006). Flowering invasive plants also add a unique spatial dimension to invasion biology, since by using local pollinators to reproduce, they can interact and compete with plants that are not in their direct proximity.

By sharing pollinators with local co-flowering plants, invasive flowering plants interact with local plant-pollinator networks, with potential implications for reproduction in native plants. A study by Memmott and Waser (2002) used historical records to determine how alien plants integrate into native plant-pollination webs. They created a web of all flower-visiting animal species and native and alien plants species for a community, and found that the alien plants were indeed well connected into native plant-pollinator network. Furthermore, Vila et al. (2009), using pollinator sampling in invaded and uninvaded plots, compared the structure of invaded and uninvaded plant-pollinator networks and found that alien species were visited on average by almost half the pollinator species present, and that in general, pollinators depended upon alien plants more than native plants.

By integrating into local pollination networks and thus sharing a potentially limiting resource, there are two general outcomes for interactions for pollination between native and invasive flowering plants: competition or facilitation (Rathcke 1983). Recent reviews indicate that the majority of studies published on invasive-native plant-pollination interactions find that competitive effects dominate (Bjerknes et al. 2007, Morales and Traveset 2009). Since the ecological mechanisms are discussed in the chapters that follow, I focus here on the long-term implications of competitive and facilitative interactions among invasive and native plant species.

In cases where natives in invaded communities consistently suffer lower male or female fitness due to competition for pollinators, there is the potential for altered selection on heritable floral traits. One possible outcome is the evolution of self-fertilization, which results in less reliance on pollinators for seed production and reduces competition (e.g., Fishman and Wyatt 1999). A second outcome could be selection for altered flowering time to reduce overlap with the competitor (e.g., Waser 1978). Third, selection for floral traits that reduce the deposition of heterospecific pollen could be favoured (e.g., Caruso 2000). Finally, in cases of severe fitness losses as a result of competition for pollinations, local extirpation or extinction are potential outcomes (Wilcock and Neiland 2002). One important point: all the aforementioned studies investigated impacts of co-flowering natives. To my knowledge, there has yet to be a published study on altered selection due to the presence of an invasive plant.

A second, and less commonly reported scenario is that the presence of an invasive plant in a community facilitates the pollinator-mediated reproduction of natives. If the presence of an invasive flowering plant in a community serves to enhance, rather than hinder fitness of natives via facilitation of pollination, one would expect selection on floral traits to increase pollinator sharing and overlap. While examples of facilitation of pollination are present in literature (e.g., Thomson 1978; Laverty 1992; Johnson et al. 2003; Moeller 2004), examples of invasives facilitating native pollination are rare (but see Molina-Montenegro et al. 2008), and studies of selection in response to facilitation, to my knowledge, are non-existent. Yet, the establishment of new populations of native species and the maintenance of existing populations could be enhanced if invasives facilitate native plant fitness. Long term monitoring of patterns of selection in communities of invasive and native species is needed to investigate these hypotheses.

STUDY SYSTEM AND RESEARCH QUESTIONS

In this thesis, I report on the results of studies designed to determine the extent of competition or facilitation for pollinators in a flowering plant community that has been invaded by *L. salicaria*. If *L. salicaria* has the potential to alter *D. verticillatus* reproduction through interactions with pollinators, the trajectory of floral selection and long term survival of populations of native *D. verticillatus* could be affected. Both species are described in detail in the two chapters that follow; I therefore keep my introductory remarks on them brief. *L. salicaria* and *D. verticillatus* are members of the same family (Lythraceae), and they share a number of floral traits such as flower colour, inflorescence shape, pollinator rewards, and approximate flower size. They have been previously observed to share pollinators (C. Eckert, *pers. comm.*), and as such, make an ideal study system to investigate invasive impacts on native pollination.

Collection and field sites were located on or near Lake Opinicon, Ontario (44.567°N 76.324°W) in shallow estuarine swamp and marshlands at the Queen's Biological Station. *D. verticillatus'* distribution around the lake is somewhat patchy, likely because it is limited by a lack of suitable habitat, which includes slow moving, shallow water. Prominent co-flowering species were few, typical of such habitat, and included *Nymphaea odorata, Pontederia cordata* and *Nuphar lutea* (formerly *variegatum*). *N. orodata* and *P. cordata* have been observed to be pollinated by some of the same fauna as *D. verticillatus*, but the extent of pollinator sharing with these species was exceeded by far by *L. salicaria* in invaded sites (Da Silva, *unpublished data*).

My research employs tests of pollen limitation, pollinator observations, and greenhouse experiments to determine the impact of *L. salicaria* on native *D. verticillatus*. Pollen limitation experiments are a means to quantify how much reproduction is limited by inadequate pollen receipt and is determined by comparing plants that are pollinated naturally with plants that have been experimentally supplemented with compatible pollen. The difference in reproduction between unsupplemented and supplemented plants gives researchers an idea of the maximum reproductive output for a plant in that system when more than enough pollen is received (reviewed in Ashman et al. 2004 and Knight et al. 2005). In my research, tests of pollen limitation provided a standard means of comparing reproduction in plants from sites that vary in their invasion status. Pollinator observations were designed to complement pollen limitation experiments, with particular focus on determining whether the quantity and identity of pollinators visiting *D. verticillatus* differed in invaded and uninvaded sites. Finally, greenhouse experiments were conducted to determine whether the transfer of *L. salicaria* pollen to *D. verticillatus* stigmas had a detectable impact on seed set in *D. verticillatus*.

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Chapter 2:

THE IMPACT OF AN INVASIVE PLANT SPECIES ON POLLINATOR-MEDIATED FITNESS IN A NATIVE: EVIDENCE FOR FACILITATION

INTRODUCTION

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

Invasive plants may negatively impact pollinator-mediated fitness in native species through two main avenues. First, by acting as a foreign pollen source in the community, invasive plants can increase the amount of heterospecific pollen transfer, leading to a decrease in pollen quality (Lopezaria-Mikel et al. 2007). Second, by usurping pollinators that may have otherwise visited native species, invasives can limit the quantity of pollen received (Waser 1983). Reductions in pollen quantity and/or quality are often associated with pollen limitation, defined as inadequate receipt of compatible pollen grains resulting in lower seed and/or fruit production (Burd 1994; Knight et al. 2005). There is a large body of literature on the prevalence of pollen limitation in a variety of systems, with one study finding that 62% of 258 species surveyed display pollen limitation (Burd 1994; Ashman et al. 2004).

If invasive plants compete with natives for pollinators and reduce the quality and/or quantity of native pollen receipt, it is possible that natives in invaded communities will suffer from increased pollen limitation and therefore reduced fitness. Most studies published on the subject indicate that invasive plants can alter pollinator visitation rates and reduce seed and/or fruit set in natives (e.g, Grabas and Laverty 1999; Brown and Mitchell 2001; Chittka and Schurkens 2001). Furthermore, the loss of reproductive opportunity due to the presence of a competitive co-flowering species can alter patterns of selection on native floral traits (Caruso 2000; Smith and Rausher 2008). However, to our knowledge, no study has explicitly compared pollen limitation of native plants in invaded and uninvaded communities.

A smaller body of research suggests that co-flowering species can facilitate one another's 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 that occur alone. Similarly, a study performed by Molina-Montenegro et al. (2008) found that after accounting for density, the attractive invasive flowering shrub *Lupinus arboerus* acted as a "magnet species" (Thomson 1978) for the less attractive invasive *Cardus pycnocephalus*, increasing pollinator visitation rates and seed output where the two species grow together compared to where *C. pycnocephalus* grows alone. If a highly attractive invasive species can act as a magnet species for native plants and facilitate their pollination by drawing more pollinators to a patch, we expect native species to suffer less pollen limitation in invaded than uninvaded communities.

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 found in Lythraceae), rely on the same pollinators (C. Eckert, *pers. comm.*), and co-flower for approximately three weeks in late summer, they provide an ideal study system in which to test the impact of an invasive species on pollinator-mediated reproductive success in a native. Our specific objective was to explore the impact of the invasive plant *L. salicaria* on pollinator-mediated fitness in its native relative *D. verticillatus.*

METHODS

Study species and sites

L. 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 (Levin 1970; O'Neil 1997; Comba et al. 1999). Interestingly, *L. 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 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 (Thompson et al. 1987; Brown et al. 2002). 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.

D. 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 tristyly, or three distinct style morphs (Graham et al. 2005). *D. verticillatus* can reproduce both sexually (via pollinator mediated pollen transfer) or clonally (via arenchymous 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 in *D. verticillatus* is costly due to the presence of inbreeding depression (Eckert and Barrett 1994) and selfing rates in populations of *D. verticillatus* in Southeastern Ontario have been shown to be around 30% (Eckert 2002). *D. verticillatus* is naturally found 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 summer 2009. Naturally occurring populations of *L. salicaria* and *D. verticillatus* were surveyed in June 2009, and from these, 5 sites were chosen for study based on similarities in size 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 2.1). Sites were chosen to ensure that they were a minimum of 200m apart, to reduce the potential for one large site with similar plant genetics and pollinator community being sampled twice.

Pollen limitation

To measure the effect of the presence of *L. salicaria* on the reproductive success of *D. verticillatus*, a pollen limitation experiment was conducted. 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), branches chosen were at least 2m apart, this being the likely distance where most clonal progeny would be found (Dorken and Eckert 2001). Additionally, to account for altered allocation between sexual and clonal reproduction in different branches, those chosen for this study were matched, as closely as possible, for the number of flower buds. To control for the potential impact of resource allocation between 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 5 haphazardly chosen branches from the same population. Donor branches were chosen at least 2m 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 5 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 were collected, pollen was mixed thoroughly and gently applied to all treatment plant stigmas with a toothpick. Supplemental pollinations were performed on all open flowers on all supplemental treatment branches twice a week throughout the entire flowering period to more closely mimic natural pollen deposition.

Once flowering was completed, *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 lab, 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, Inc., http://www.mathworks.com). Seeds per fruit were then averaged for all fruits from the same branch to provide one mean seeds per fruit value for each branch.

Pollinator observations

To explore the underlying mechanisms of pollen transfer to *D. verticillatus* in invaded and uninvaded populations, pollinator observations were conducted in each of the 5 sites. Observations were conducted in a 6 x 3m permanently cordoned-off plot in an area of each site where plant density matched that of the other sites. Each observation period was conducted with 3 observers placed at designated locations around the plot, such that the entire plot could be observed from at least 1 of the 3 vantage points. Flowering density of *D. verticillatus* and *L. salicaria* within plots was recorded prior to each observation period as number of flowering branches or inflorescences. Observations were conducted for 30 minutes twice a week. Each observer located a pollinator within their "vantage area" and followed it throughout its foraging bout within the plot. 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. Each visit to a flower or branch was recorded. Once a pollinator was lost or left the plot, observers scanned the plot until a new pollinator was located. Records were kept using digital voice recorders that were later transcribed. To measure visitation rate in a way that could be compared across sites irrespective of flowering phenology and density within plots, our data were converted to # visits per branch per hour for each site. *D. verticillatus* flowering density was also measured across sites using two randomly located 2x2m quadrats in each site, twice a week. Density was counted as the number of flowering *D. verticillatus* branches within each 2x2m quadrat.

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. A fully specified ANOVA model was run using JMP 8.0.2 (SAS Institute 2009) and estimated mean squares were used to create F ratios, with each factor being tested for the appropriate denominator for such an analysis (Quinn and Keough 2002). Visitation rate data were analyzed using a nested model with invasion status and site nested within invasion status. Analyses for all data were performed on S-PLUS 8.0 (Insightful Corp 2007) or JMP 8.0.2 (SAS Institute 2009).

RESULTS

Pollen limitation

Of the 150 branches treated in total over the 5 sites, 23 were not counted due to loss through herbivory, a 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 2.2) and this effect differed by invasion status with supplemented branches setting significantly more seed than open branches in the uninvaded populations (Fig. 2.1, Table 2.2). Site nested within invasion status neared significance, but we had no hypotheses of interest related to this factor, and accordingly do not discuss any implications of it here. All other model effects were non-significant (Table 2.2).

Pollinator observations

Using the *D. verticillatus* flowering density counts taken in plots prior to each observation period, visitation rate data were converted to # visits per branch per hour for analysis, with one value for each observation period at each site. Our nested model revealed significant differences in visitation rate to branches by site (F=4.15, df=3, p=0.00158) but no significant difference by invasion status (F=0.347, df=1, p=0.597). To ensure that any trend observed could not be attributed to differences in *D. verticillatus* flowering density, density was measured twice a week throughout the flowering season at each site. After square-root transforming to meet the assumptions of ANOVA, we found no significant difference in average flowering branch density across sites (F=1.35, df=4, p=0.265).

DISCUSSION

We found that *D. verticillatus* branches at sites invaded by *L. salicaria* suffered less pollen limitation than branches at sites that were not invaded, suggesting that the presence of the invasive plant *L. salicaria* facilitates pollination in the native plant *D. verticillatus*. While we expected that the underlying mechanism behind this decreased pollen limitation would be explained by an increase in the visitation rate to *D. verticillatus* branches in invaded sites, we found no difference in visitation rates between invaded and uninvaded populations.

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 movement patterns (e.g., Chittka and

Schurkens 2001, Lopezaria-Mikel et al. 2001). While a few have measured impacts on reproduction, we could find no other studies that conducted tests of pollen limitation. Pollen limitation experiments are crucial to our understanding of the impact of plant invasions because they can uncover what simple observations of pollinator visitation patterns or uncontrolled measurements of seed set cannot - the difference between actual seed set and the seed set that would be achieved if pollinators were not limiting (Burd 1994). These comparisons allow us to assess the level of pollen transfer in different populations, and if pollen limitation is found to vary consistently according to invasion status, it can provide evidence of an impact of invasion on pollinator-mediated fitness in native species.

Additionally, most studies that have used other means to examine the influence of invasive plants on pollinator-mediated fitness in native species have found a negative impact. For example, a recently published meta-analysis of alien vs. native impacts on co-flowering native plants found predominantly detrimental impacts of alien plants on pollination and reproduction of natives, particularly when the alien and native have similar floral symmetry or colour (Morales and Traveset 2009). This echoes a review paper published by Bjerknes et al. (2007) which found that four of the five experimental studies they summarized showed reduced visitation or seed set as a result of the presence of the alien, while the last found no effect. Interestingly, of the 10 observational and experimental studies they reviewed, none found that alien species increased seeds per fruit set in natives. Moreover, all other previous 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).

Indeed, evidence for facilitative pollinator-mediated interactions between invasive and native plants are rare. In a literature search, we encountered only two published reports of an invasive plant facilitating reproduction, and both studies found facilitation only under specific conditions. Nielsen et al. (2008) found that the invasive species *Heracleum mantegazzianum* enhanced pollinator visitation to *Mimulus guttatus* when in close proximity, but this effect did not translate into seed set increases. Munoz and Cavieres (2008) demonstrated that in pollinator-scarce alpine systems, the presence of the invasive *Taraxcum officinale* increases seed output in *Hypochaeris thrincioides,* but only when *T. officinale* density was limited to a single individual.

Our observations of pollinator movement in invaded and uninvaded communities were designed explain how the presence of invasive *L. salicaria* could impact pollination in *D. verticillatus*. When we measured visitation as visits per branch per hour, visits to *D. verticillatus* did not differ between invaded and uninvaded communities (*F*=0.3469, df=1, *p*=0.5968). A post-hoc analysis on our data revealed that the achieved power of this study to detect differences in visitation rates by invasion status was very low (Power = 0.07). It is then possible that these differences did exist, but could not be detected given our low number of replicate sites. Given the effect sizes observed in this study, we calculated that we would need between 20 and 30 sites of each invasion status to achieve approximately 50% power, suggesting that many more sites would be needed to accurately detect differences in visitation rates. However, intriguingly, when we examined our data on a finer scale, using the number of flowers visited per branch per pollinator, and pollinators rather than sites as replicates, we do find a trend towards more flowers visited per branch in invaded sites (*F*=5.6283, df=1, *p*=0.0954). Noting that *D. verticillatus* is self-compatible in this part of its range (Eckert 2002), this shift in pollinator foraging patterns could provide an explanation for why *D. verticillatus* branches in invaded sites are less pollen limited: if pollinators in invaded sites make more within-branch movements, this could lead to an increase in selfing via geitonogamy (the movement of self pollen among flowers of the same plant) (Lloyd and

Schoen 1992). Since selfing is known to be costly in this species (Eckert and Barrett 1994), a shift towards more geitonogamy in invaded sites has the potential to be costly for *D. verticillatus*.

We acknowledge that there could be other factors influencing pollen limitation in invaded sites. Haig and Westoby (1988), later revisited by Ashman et al. (2004), propose that seed set is not only limited by adequate pollen capture, but also by 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). Even if branches chosen from at least 2m apart were from the same plant and *D. verticillatus* were shifting resources from open to supplemented branches, we have no reason to suspect that the magnitude of this effect would differ between invaded and uninvaded sites and alter the interpretation of our results.

One strength of our study is that we conducted our tests of pollen limitation on plants in natural communities. Because of this, we are confident that our results are indicative of the processes occurring within the communities that we are interested in. However, certain drawbacks are also associated with this approach. Since we did not experimentally introduce *L. salicaria* into previously uninvaded populations, there could be other confounding site differences that correspond with invasion status such as differences in the pollinator community or differences in population style morph ratios. However, we found no notable site differences in pollinator community composition (Table 2.1) and 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).

Future studies investigating impacts of invasive species on pollination and reproductive success in natives could control for potential site differences by using pollen limitation studies on plants that can be matched for life history. Using half-sibs in plots taken to both invaded and uninvaded populations or experimental introductions of *L. salicaria* into previously uninvaded populations would allow for a relatively easy assessment of pollen limitation and impacts of invasion while accounting for other issues associated with using naturally invaded communities. On the other hand, ecological concerns likely trump the potential advantages of such an experimental design. Additionally, future work should explore the altered pollinator foraging patterns detected in invaded sites and investigate the potential for increased geitonogamy in invaded sites.

CONCLUSIONS

We conducted tests of pollen limitation in naturally occurring populations of *D. verticillatus* to determine if the invasive *L. salicaria* affects pollinator-mediated reproduction. Contrary to our expectations of a competitive interaction, our findings support a facilitative effect of invasion on the reproduction of the co-flowering native species. Moreover, our results could not be explained by increased visitation to branches of the native species in the presence of the invasive. Intriguingly, our findings suggest that in the presence of the invasive, differences in the way pollinators forage after arriving on a native plant could be responsible for alleviated pollen limitation. However, further study is needed to fully elucidate the mechanism underlying our findings. In the interim, our study adds an important contrasting view to the predominantly competitive body of research on invasive impacts on pollination, and has complex and interesting implications for the ecological and evolutionary impacts of biological invasions.

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Table 2.1. Details on *D. verticillatus* sites used in this study.

Site	*L. salicaria*	Flowering density	Pollinators						Morph (%)
			B	A	L	So	Sy	W	
Cow Island Marsh	invaded	17.7 (±2.6)	*X*	*X*	*X*	*X*	*X*	*X*	7 L 92 S
Deadlock Bay	uninvaded	23.8 (±10.7)	*X*		*X*	*X*	*X*	*X*	76 L 23 S
Indian Lake	invaded	43.3 (±11.9)	*X*	*X*	*X*	*X*	*X*	*X*	24 L 65 M 10 S
Little Island	uninvaded	24.8 (±4.7)	*X*		*X*	*X*	*X*	*X*	65 L 34 S
Silo Bay	invaded	21.5 (±7.8)	*X*		*X*	*X*	*X*	*X*	41 L 58 S

Note: Flowering density measured as mean number of flowering *D. verticillatus* branches within a $2m^2$ plot, shown here as mean (± SE). Abbreviations for pollinators: B = *Bombus*, A = *Apis mellifera*, L = Lepidopteran, So = solitary bee, Sy = Syrphid fly, W = wasp. Morph percentages were calculated using proportions of treatment branches in a site, chosen before flowering, L = long styled, M = mid styled, S = short styled morph.

Table 2.2. Results of a partly nested split-plot ANOVA of seed set in *D. verticillatus* in sites with or without *L. salicaria* invasion.

Source	df	SS	*F*	*P*
Invasion[1]	1	46.337	0.2855	0.6285
Treatment[2]	1	250.916	10.2562	0.0292*
Invasion*Treatment[2]	1	185.858	7.5969	0.0467*
Site(Invasion)[3]	3	499.226	7.7809	0.0630
Treatment*Site(Invasion)	3	64.1605	0.2559	0.8570
Error	117	9778.592		

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

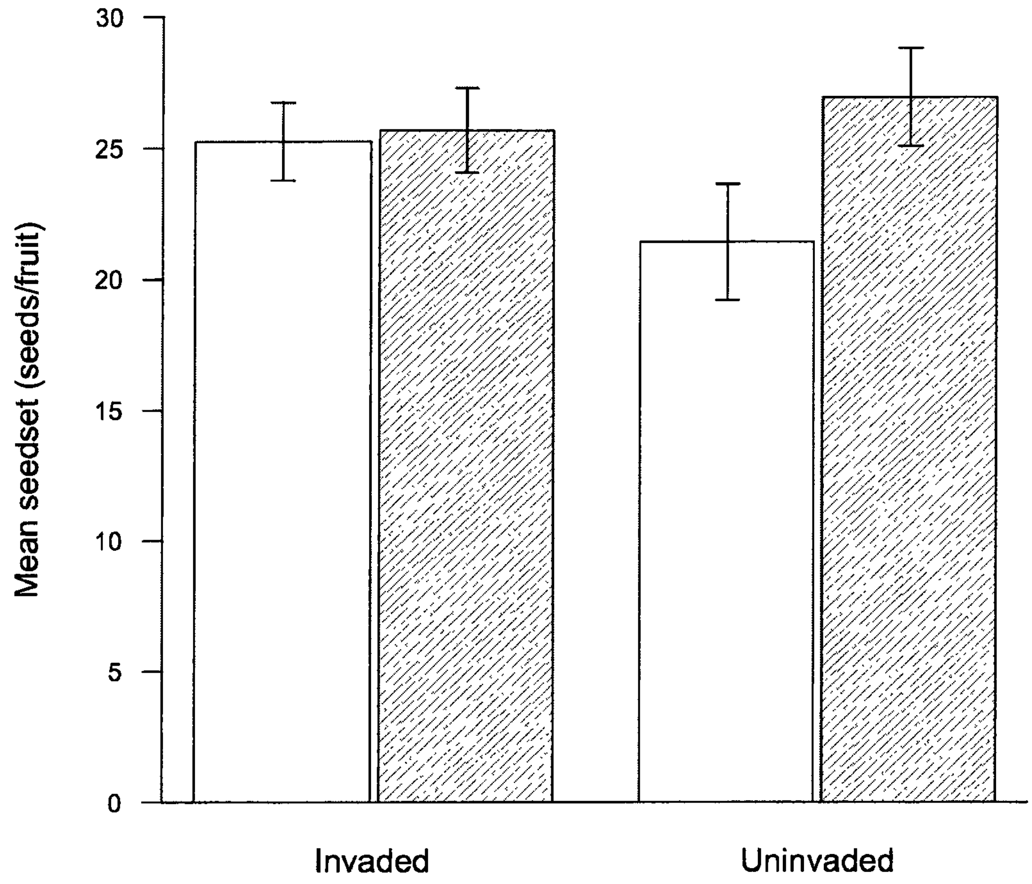
[1] Tested over 0.9505*Site(Invasion) + 0.0495*Residual

[2] Tested over 0.9505Treatment*Site(Invasion) + 0.0495*Residual

[3] Tested over Treatment*Site(Invasion)

* denotes statistical significance at the 0.05 level.

Figure 2.1. Least mean squares ± 2SE of *D. verticillatus* seed set (seeds per fruit) for open and supplemented treatment branches in invaded and uninvaded communities (open bars = open pollinated branches, shaded bars = supplementally pollinated branches).



Chapter 3:

THE EFFECT OF INVASIVE POLLEN DEPOSITION ON SEED SET IN A NATIVE PLANT

INTRODUCTION

Insect-pollinated plants rely on the successful transfer of pollen from male reproductive parts (anthers producing pollen) to female reproductive parts (receptive stigmas) for fertilization to occur. The presence of a novel flowering plant species in a community can alter local pollinator movement (Lopearaiza-Mikel et al. 2007), and, as such, has the potential to affect pollen transfer and native fitness (reviewed in Bjerknes et al. 2007 and Morales and Traveset 2009). Yet, little attention is paid to the mechanisms underlying such effects. Specifically, few studies have directly measured the impact of invasive 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 of natives (Waser 1983). In the case of reduced pollen quantity, highly attractive invasive plants can draw pollinators from natives, resulting in less conspecific pollen transfer, lower seed set, and the potential for lower fitness (e.g. Chittka and Schurkens 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 (Matsumoto et al. 2010, reviewed in Morales and Traveset 2008)

In order for heterospecific pollen deposition to occur, certain criteria must be satisfied. The two species must co-flower, 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 both species of pollen are transferred to a 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 the germination of conspecific pollen tubes through blocking the native stigma or style (Galen and Gregory 1989). A second scenario is that heterospecific pollen can chemically interfere 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). A final possibility is that heterospecific pollen can successfully germinate on the stigma and fertilize ovules, resulting in the formation of hybrids, 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 (Memmott and Waser 2002; Vilá et al. 2009) and that alien pollen grains tend to dominate native pollen transfer webs (Lopezaraiza-Mikel et al. 2007). Additionally, researchers have found examples of pollinators switching frequently between native and invasive species (Brown et al. 2002; Grabas and Laverty 1999), making it likely that natives in invaded communities could experience heterospecific pollen transfer. Finally, since invasive species, by definition, are newly present in communities, it is likely that natives have not had adequate time to evolve responses to any consequences of invasive pollen deposition, making them excellent systems to examine mechanisms and measure selection (Jakobsson et al. 2008).

The few studies that specifically investigated heterospecific pollen deposition in relation to invasive species have found mixed effects of alien pollen on native seed set, ranging from negative to neutral. Previous work done on interactions between the invasive *Taraxacum officinale* and native relative *Taraxacum japonicum* confirms that supplemental hand-pollination treatments of mixed invasive/native pollen reduced seed set over conspecific only pollen (Matsumoto et al. 2010). In contrast, controlled hand pollination experiments using invasive *Carpobrotus* spp. pollen on native plants species on the Balearic Islands found that invasive pollen did not interfere significantly with seed set in natives (Moragues and Traveset 2005), a result similar to work done by Tscheulin et *al.* (2009) where native *Glaucium flavum* was hand pollinated with invasive *Solanum elaeagnifolium* pollen, also resulting in no reductions in seed set. These mixed results suggest that more study is needed into invasive-native interactions for pollinators and that the consequences of heterospecific pollen deposition could be species and context dependent.

In this study, we were interested in exploring the mechanisms of pollinator sharing between the showy invasive wetland plant, *Lythrum salicaria* L. (Lythraceae) and *Decodon verticillatus* (L. Elliot). These two species are an ideal choice for a study on effects of heterospecific pollen deposition since both occur and co-flower in invaded wetlands of Southeastern Ontario, share many floral traits, have previously been observed to share pollinators (Eckert, *pers. comm.*) and are found in the same family (Lythraceae). This suggests that it is likely that *L. salicaria* pollen is transferred to *D. verticillatus* stigmas in invaded sites, with the potential for negative impacts for fitness for the native. Yet, recent related work has shown that *L. salicaria* alleviates pollen limitation in *D. verticillatus* (Chapter 2, Da Silva et al. *in review*). Interestingly, this suggests a facilitative, rather than competitive, interaction between the two species and hints that negative impacts of heterospecific pollen deposition may not exist between these two species. However, similar work conducted between *L. salicaria* and another co-flowering relative, *L. 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). This presents us with an interesting puzzle that motivates this study. It is possible that the impact of invasive *L. salicaria* pollen deposition depends on the native species that it interacts with; *L. alatum* is more closely related to *L. salicaria* than *D. verticillatus*, and the genetic similarity between the two *Lythrum* co-geners may make them more likely to interfere with one another (see Duncan and Williams 2002 and Strauss et al. 2006 for contrasting opinions on relatedness and invasiveness). Alternately, it is possible that the impacts of invasive pollen deposition are negative for both *L. alatum* and *D. verticillatus*, which makes the facilitative result of the related pollen limitation study (Da Silva et al. *in review*) all the more intriguing.

To fully elucidate the impacts of *L. salicaria* on *D. verticillatus* and to determine if the facilitative effect observed in related studies is, in part, due to a neutral, rather than a negative impact on invasive pollen deposition, we undertook a greenhouse pollination study. Specifically, we conduct supplemental hand pollinations on *D. verticillatus* with conspecific and mixed invasive/conspecific pollen to determine whether the addition of invasive pollen interferes with the ability of conspecific pollen to set seed. If negative impacts of *L. salicaria* pollen are observed, it suggests that heterospecific pollen transfer could be detrimental for *D. verticillatus in situ*, and challenges us to explain the facilitation result of our related study (Da Silva et al., *in review*). Alternately, if no negative effects of *L. salicaria* pollen deposition are found for *D. verticillatus,* then this, taken together with the related aforementioned study with *L. alatum* (Brown and Mitchell 2001), suggests that the negative impacts of *L. salicaria* pollen transfer may instead depend on the evolutionary distance between invasive-native species pairs.

METHODS

Study species

L. 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 generalist pollinator requirements (Levin 1970; O'Neil 1997; Comba et al. 1999). *L. 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 nineteenth century, *L. salicaria* has attracted considerable attention from wetland ecologists and conservation biologists due 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 semi-natural systems provides a unique opportunity to study the effects of an alien invasive in a variety of communities and pollination contexts.

D. 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 tristyly, or three distinct style morphs (Graham et al. 2005). *D. verticillatus* can reproduce both sexually (via pollinator mediated pollen transfer) or clonally (via arenchymous 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 due to the presence of elevated levels of inbreeding depression (Eckert and Barrett 1994). *D. verticillatus* is naturally found 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.

Pollen addition experiment

Seeds from six *D. verticillatus* populations (three *L. salicaria* invaded, three uninvaded) were collected from the Queens University Biological Station (44.567°N, 76.324°W) and the surrounding area in September and October 2008. Seeds were cold stratified for three 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 4" pots and grown to flower in a glasshouse at the University of Ottawa under controlled temperature and light (temperature ranged from 22 – 30°C and 16h light was maintained by artificial lighting). Plants were watered daily and fertilized bi-weekly with 20-20-20 fertilizer diluted to 2g/L. Fifty-three *D. verticillatus* plants reached flowering, and of these, thirteen were randomly assigned as pollen donors. The remaining forty were randomly assigned to one of two groups: the pure native pollen control group or the invasive mixed pollen treatment group. Plants in the native 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 50:50 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 ensures 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 two 1.5mL microcentrifuge tubes, one for each treatment. The tubes were placed under a 60W incandescent blub for approximately 20 minutes 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 four 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 two 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. Due to the extremely close proximity of two uninvaded sites plants sourced from both of these sites were grouped together and analyzed as if from one site, changing the number of sites modeled from six to five. A partly-nested ANOVA was used to determine if seed set differed across pollination treatments, taking into account variation by site. Invasion status was also included in the model in order to investigate the potential for a response to treatment to differ based on the presence of *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 2007). Between assumptions were met for normality and homoscedasticity (Shapiro-Wilk Goodness-of-fit, p=0.8053, Levene, p=0.2946). Since treatments were assigned randomly within each site nested within invasion status, we are confident that within assumptions were met (Quinn and Keough 2007). Statistical analyses were performed on S-PLUS 8.0 (Insightful Corp 2007) or JMP 8.0.2 (SAS Institute 2009).

RESULTS

Three *D. verticillatus* plants died prior to or shortly after flowering and were excluded from the study, and 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).

Partly nested split-plot analysis of seed set revealed significant differences by treatment, with all other factors being non-significant (Fig. 3.1).

Additional data collected on date of first flowering was analyzed using a non-parametric Kruskall-Wallis rank sum test. Differences in first flowering time were not significantly different when modeled by site alone (Kruskall-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 by invasion status (*F*= 1.7290, df=4, *p*= 0.1570; *F*= 0.3736, df=1, *p*=0.5436, respectively).

DISCUSSION

Using pollen addition experiments conducted in greenhouses, we found that seed set in the native plant, *D. verticillatus*, was lower when hand-pollinated with a mixture of conspecific and invasive pollen than with pure pollen alone. This demonstrates that the application of *L. salicaria* pollen interferes with the ability of *D. verticillatus* pollen to set 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.*

Brown and Mitchell (2001) found 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 the pure invasive treatment 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 this 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. 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 which of these mechanisms are responsible for reduced mixed seed set will require further experimentation.

This result has interesting implications when considered along with previous work done in this system. This work 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 coflowering relatives. Interestingly, the similarities do not extend to pollen transfer *in situ.* Our field study in the *D. verticillatus* system found that invaded sites suffer from less pollen limitation than uninvaded sites, suggesting a lack of negative impact of invasive pollen deposition on fitness in *D. verticillatus* (Chapter 2, Da Silva et al. *in review*). At the same time, field array experiments conducted with *L. alatum* support the finding of a negative impact of the invasive 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.

Relating this study to others conducted with natives outside of Lythraceae, our research adds more evidence for negative, rather than neutral, effects of invasive pollen deposition (see Matsumoto et al. 2010 for negative, Moragues and Traveset 2005 and Tscheulin et al. 2009 for neutral effects). 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. The Moragues and Traveset (2005) study, for example, suggests that one of the reasons for the lack of negative impact of invasive pollen observed on native stigmas is due to the fact that the species are not generally 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, of the other studies we found that conducted mixed invasive hand pollinations on native plants, those that had negative impacts were conducted between relatives (Matsumoto et al. 2010) and those that found neutral effects were conducted between co-flowering non-relatives (Moragues and Traveset 2005 and Tscheulin et al. 2009), suggesting that large differences in relatedness could determine impacts of supplemental invasive hand pollinations. Another possibility for the range of effects observed in studies of invasive pollen impacts on natives is the timing of invasive pollen deposition. In their study involving native two 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). In order to fully elucidate the impact of invasive pollen deposition on natives, more studies that use a range of invasive and native species combinations and a variety of types and timings of hand pollinations are needed.

If the deposition of heterospecific pollen on native species reduces seed set consistently, selection could favour traits that mitigate these fitness losses. 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 selfing) 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 (e.g., Waser 1978; Aizen and Vázquez 2006; Caruso 2000, Fishman and Wyatt 1999), studies on the selection of native phenotypes as a result of new species invasions are, to our knowledge, non-existent. Our study, and others like it, are taking the first step to identifying whether invasive species can alter selection on floral traits in native species. By identifying competition at the level of the stigmatic surface, research can now move forward to determine whether this competition has implications for selection.

Our experiment included invasion status in our model since we had expected that we might observe evolved differences in *D. verticillatus* plants sourced from communities that have faced selection due to heterospecific pollen deposition. For example, if selection in invaded sites had favoured plants that were better suited to contend with heterospecific pollen, we would have found 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 3.1). This result is not entirely surprising. First, there could 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 exist in 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 potential implications for selection and evolutionary responses to invasion. This research adds to an increasing amount of evidence for an impact of invasive plants on native pollination systems, and raises interesting questions about the potential for natives to evolve protective mechanisms against these threats.

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Table 3.1. Results of a partly nested split-plot ANOVA of seed set in *D. verticillatus* in pure and mixed pollination treatments.

Source	df	SS	*F*	*P*
Invasion[1]	1	136.391	0.7889	0.4416
Treatment[2]	1	853.559	11.6799	0.0465*
Invasion*Treatment[2]	1	206.165	2.8211	0.1980
Site(Invasion)[3]	3	494.077	2.3202	0.2537
Treatment*Site(Invasion)	3	212.949	1.8308	0.1654
Error	27	1046.8532		

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

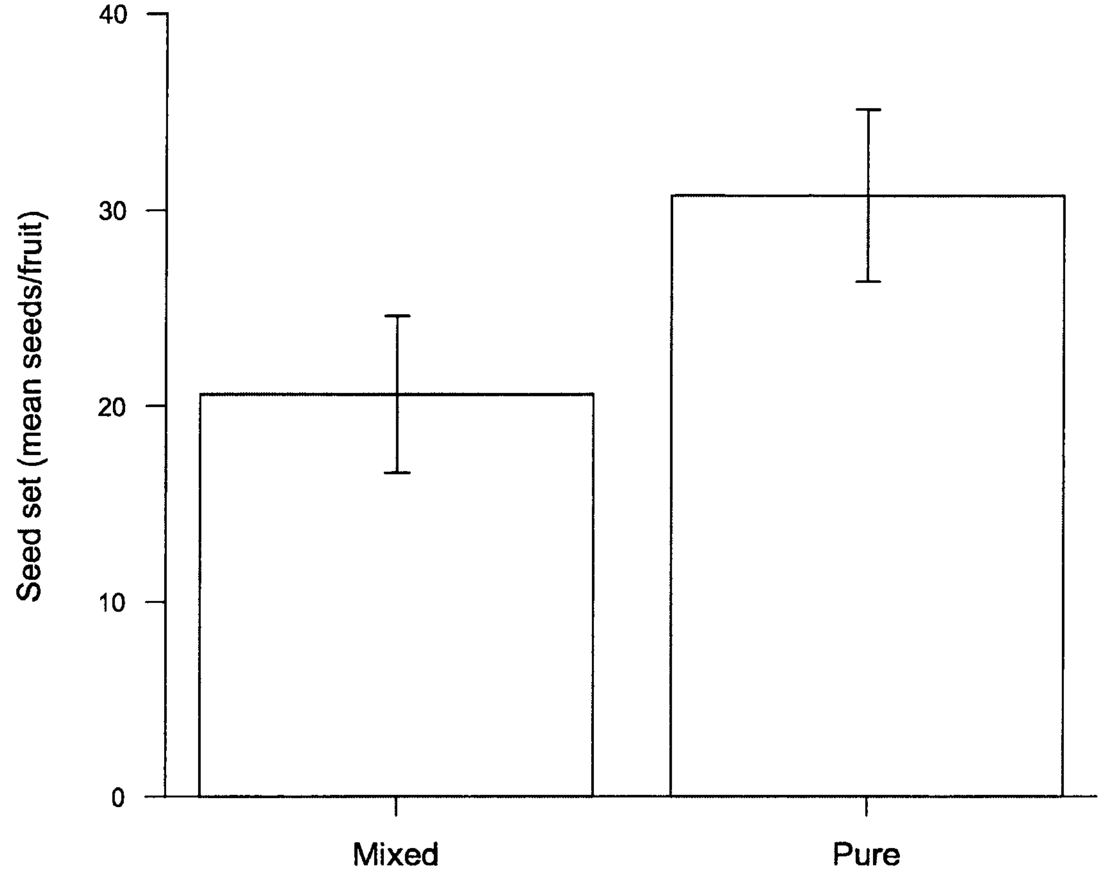
[1] Tested over 1.0651*Site(Invasion) - 0.065*Residual

[2] Tested over 1.0651*Treatment*Site(Invasion) - 0.065*Residual

[3] Tested over Treatment*Site(Invasion)

* denotes significance at the 0.05 level.

Figure 3.1: Least mean squares of *D. verticillatus* seed set (seeds per fruit) ± 2 SE in mixed invasive-native pollen and pure native pollen hand supplementations



Chapter 4:

DISCUSSION AND FUTURE DIRECTIONS

In this thesis I have described the results of an investigation into the pollinator-mediated impacts of the invasive plant species, *L. salicaria,* on reproduction in the native species, *D. verticillatus*. In the beginning, I discussed the prediction that pollinator-mediated interactions between these two species could be predominantly competitive or facilitative. My experimental test of pollen limitation in Chapter 2 demonstrates that the presence of *L. salicaria* in a community reduces pollen limitation in *D. verticillatus*, suggesting facilitation. Negative effects of invasive pollen deposition, however, were observed in greenhouse experiments reported in Chapter 3, which would typically point to competition. Additionally, observations of pollinator visitation rates in the field found no difference in number of visits per branch in invaded and uninvaded sites, but a preliminarily investigation suggests that instead, more flowers were visited per branch in invaded sites. This suggests the potential for a neutral or negative effect of *L. salicaria*, and, if taken in isolation, could be indicative of increased geitonogamy (the movement of self pollen among flowers on the same plant) in invaded sites and could potentially lead to a decrease in seed fitness through post-fertilization processes (Harder and Barrett 1995 and de Jong et al. 1993).

Relating these results to each other proves to be interesting and challenging. If further exploration into geitonogamous movement on branches corroborates our initial findings, it is possible that the perceived facilitative effect of *L. salicaria* alleviating pollen limitation is actually an increase in the production of low quality selfed seeds. This could be tested through germination of seeds collected in the field. If germination tests reveal that seeds from invaded sites are equally as fit as seeds from uninvaded sites, I could more confidently conclude that the presence of *L. salicaria* has a beneficial effect on fitness in *D. verticillatus* and that facilitation is actually occurring. On the other hand, if seeds from invaded sites are less fit than seeds from uninvaded sites, it suggests that geitonogamous movement by pollinators is producing more seeds but of a lower quality, which has the potential to negate the fitness benefits of increased seed production. Interestingly, seeds collected from 3 invaded and 3 uninvaded sites that were germinated to grow treatment plants for greenhouse research revealed no significant difference in germination rates by invasion status (F= 0.2161, DF=1, p= 0.6662), suggesting that seeds in invaded sites do not suffer greater inbreeding depression, or do but with effects that are only observed at a later stage of development (i.e., late-acting inbreeding depression). This germination test was also conducted using seeds from 2008 collections, and to be truly informative for this research the test should be repeated with seeds collected from 2009, when the facilitation result was observed. Regardless, I am still tasked with rectifying the finding of facilitation in the field study with the pollinator observations and greenhouse data.

Supplemental work conducted on pollinator movements in invaded sites confirmed that pollinators frequently move between *L. salicaria* and *D. verticillatus* (V. King, *unpublished*). Since pollinators are moving between the two species and the Chapter 3 results indicate that the deposition of *L. salicaria* pollen on *D. verticillatus* is detrimental to seed set, we would expect seed set in invaded sites to be reduced, not facilitated. There are a number of potential explanations for a lack of a negative effect of pollinator movement between species. First, pollinators in the field may not transfer the same amount of invasive pollen as was deposited on *D. verticillatus* stigmas in the greenhouse experiments. Simple swabs and counts of pollinator pollen loads in the field and supplementary greenhouse experiments with altered ratios of invasive: native pollen in the mixed treatment could be performed in order to explore this hypothesis further. Alternatively, it is possible that pollinators are carrying large loads of both species of pollen, but on different parts of the body. Research in other systems suggests that increased precision of pollen placement on pollinators can result in reduced interspecific pollen transfer (Brantjes 1982, Maad and Nielsson 2004). Despite similarities in flower morphology between the two plant species, it is possible that subtle differences in anther position or certain combinations of morph type in both species could result in high heterospecific pollen loads on pollinators but low invasive pollen deposition on *D. verticillatus*. Methodology exists for determining where pollinators carry different species of pollen, the most common being methodical examination or swabbing of each region of an insects integument (Kearns and Inouye, 1993). Alternately, *D. verticillatus* stigmas could be examined under microscope to count numbers of deposited conspecific and heterospecific pollen grains, since pollen from the two species are morphologically different enough to be readily identified under a compound microscope (Da Silva, *unpublished data*).

FUTURE DIRECTIONS

Future research in this system should investigate the potential for differences in inbreeding depression in *D. verticillatus* in invaded and uninvaded sites and examine pollen placement and deposition on pollinators and *D. verticillatus* stigmas. Additionally, conducting transplant or array experiments in invaded and uninvaded sites would allow for increased control over genetic and spatial differences among invaded and uninvaded populations of *D. verticillatus*. Finally, all of the aforementioned experiments quantify female fitness. Despite difficulties associated with ease and cost of experimental techniques, future work could explore the potential the impact of *L. salicaria* on male fitness through direct (genetic markers) or indirect (number of pollen grains removed and deposited) measures (Klinkhamer et al. 1994, Sutherland and Delph 1984).

This thesis examined the pollinator-mediated impact of an invasive flowering plant on a closely related native, and demonstrates that the presence of an invasive alters seed set in natives, with the potential for positive implications on native fitness. This work highlights the importance of pollinator-mediated interactions in invaded communities, and stresses that the impacts of invasives on natives may not always be negative. Finally, this thesis opens many avenues for new research in this system and raises many interesting questions about pollinator-mediated interactions between these two species.

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